Eesti Maaülikool
Estonian University of Life Sciences

MATURITY PARAMETERS OF GRAPEVINE (*VITIS* SP) YIELD

VIINAPUU (*VITIS* SP) SAAGI KÜPSUSNÄITAJAD

MARIANA MAANTE-KULJUS

A thesis
for applying for the degree of Doctor of Philosophy
in Agriculture

Väitekiri
filosoofiadoktori kraadi taotlemiseks
põllumajanduse erialal

Tartu 2021

Eesti Maaülikooli doktoritööd

Doctoral Theses of the Estonian University of Life Sciences

Eesti Maaülikool
Estonian University of Life Sciences

MATURITY PARAMETERS OF GRAPEVINE (*VITIS* SP) YIELD

VIINAPUU (*VITIS* SP) SAAGI KÜPSUSNÄITAJAD

MARIANA MAANTE-KULJUS

A thesis
for applying for the degree of Doctor of Philosophy
in Agriculture

Väitekiri
filosoofiadoktori kraadi taotlemiseks
põllumajanduse erialal

Tartu 2021

Institute of Agricultural and Environmental Sciences
Estonian University of Life Sciences

According to verdict No 6-14/4-2 of April 19, 2021, the Doctoral Committee of the Agricultural and Natural Sciences of the Estonian University of Life Sciences has accepted the thesis for the defence for the degree of Doctor of Philosophy in Agriculture.

Opponent: **Associate professor Kaspars Kampuss**, PhD
Faculty of Agriculture
Latvia University of Agriculture

Supervisors: **Professor Kadri Karp**, PhD
Institute of Agricultural and Environmental Sciences
Estonian University of Life Sciences

Associate professor Leila Mainla, PhD
Institute of Agricultural and Environmental Sciences
Estonian University of Life Sciences

Ele Vool, PhD
Institute of Agricultural and Environmental Sciences
Estonian University of Life Sciences

Defence of the thesis: Estonian University of Life Sciences, in web, Tartu on May 24, 2021 at 13.15.

The language of current thesis was revised at Estonian University of Life Sciences Language Centre. The English language was revised by Irina Koksharova and the Estonian by Urve Ansip.

Publication of this thesis is supported by the Estonian University of Life Sciences.

ISSN 2382-7076
ISBN 978-9949-698-74-5 (trükis)
ISBN 978-9949-698-75-2 (pdf)

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following research papers, which are referred to by their Roman numerals (I–IV in the text). Papers are reproduced by kind permission of the journals concerned.

I **Maante, M.**, Vool, E., Rätsep, R., Karp, K. 2015. The effect of genotype on table grapes soluble solids content. *Agronomy Research* 13(1): 141−147.

II **Maante-Kuljus, M.**, Vool, E., Mainla, L., Starast, M., Karp, K. 2019. Berry quality of hybrid grapevine (*Vitis*) cultivars grown in the field and in a polytunnel. *Agricultural and Food Science* 28(3): 137−144. doi:10.23986/afsci.76822

III **Maante-Kuljus, M.**, Rätsep, R., Mainla, L., Moor, U., Starast, M., Põldma, P., Karp, K. 2019. Technological maturity of hybrid vine (*Vitis*) fruits under Estonian climate conditions. *Acta Agriculturæ Scandinavica, Section B - Soil & Plant Science* 69(8): 706−714. doi:10.1080/09064710.2019.1641547

IV **Maante-Kuljus, M.**, Rätsep, R., Moor, U., Mainla, L., Põldma, P., Koort, A., Karp, K. 2020. Effect of vintage and viticultural practices on the phenolic content of hybrid winegrapes in very cool climate. *Agriculture*, 10(5): 169. doi:10.3390/agriculture10050169

The contributions of the authors to the papers:

Paper	Idea, study design	Data collection/ analyses	Data analysis	Manuscript preparation
I	KK EV	**MK** EV	**MK** EV	**MK** EV
II	KK EV	**MK** EV MS	**MK** KK	**MK** LM KK
III	KK PP	**MK** RR MS	**MK** PP KK	**MK** RR LM KK UM
IV	KK **MK**	**MK** RR AK	**MK** UM PP	**MK** RR UM LM

MK – Mariana Maante-Kuljus; KK – Kadri Karp; LM – Leila Mainla; EV – Ele Vool; RR – Reelika Rätsep; UM – Ulvi Moor; PP – Priit Põldma; AK – Angela Koort; MS – Marge Starast.

ABBREVIATIONS

ACC	Total anthocyanin content
ACC_{spec}	Total anthocyanin content spectrophotometrically
ACC_{HPLC}	Total anthocyanin content chromatographically
ANOVA	Analysis of variance
FW	Fresh weight
$LSD_{0.05}$	Least significant difference
SSC	Soluble solids content
TAC	Organic acids content
TAC_{titr}	Organic acids content titrimetrically
TAC_{spec}	Organic acids content FT-IR spectroscopy
TPC	Total phenolic content
TA	Tartaric acid
MA	Malic acid
HI	Heliothermal Index
BBCH	Phenological growth stage identification scale
RH	Relative air humidity
SAT	Sum of active temperatures
Site 1	Tartu County field
Site 2	Tartu County high polyethylene tunnel
Site 3	Pärnu County high polyethylene tunnel
RF	Radiation flux
DPPH	2.2-diphenyl-1-picrylhydrazyl
HPLC	High performance liquid chromatography
Dp	Delphinidin-3-O-glucoside
Cy	Cyanidin-3-O-glucoside
Pt	Petunidin-3-O-glucoside
Pn	Peonidin-3-O-glucoside
Mv	Malvidin-3-O-glucoside
PCA	Principal component analysis
BW	Berry weight
NBC	Berries per cluster
CW	Cluster weight
FFP	Frost free period
Prec	Sum of precipitation

1. INTRODUCTION

In Northern climate, great interest has been expressed to establishing vineyards and wineries. Consequently, in 2006, the first International Viticulture and Enology Conference (VitiNord) was held in Latvia (VitiNord 2018). The VitiNord conference takes place every three years, with the location alternating between North America and Europe. This conference focuses on research and practices used in Northern vine cultivation climates. Their mission is to promote the advancement of viticulture and enology in northern environments characterized by cool or short summers and/or cold winters.

The first grapevine researcher in Estonia was plant physiologist professor Heigo Miidla. He started vine research in the middle of the 20th century. In 1964, a book on viticulture and the results of research has been published (Miidla 1964). Moreover, pomology teacher Jaan Kivistik in Räpina School of Horticulture promotes the vine cultivation. He has published books on cultivation of vines and cultivars grown in Estonia (Kivistik 2006, 2012, 2017).

Even though viticulture is increasing in its popularity, still little science-based information exists about cultivation techniques and fruit quality attributes in Estonian climatic conditions. In 2003, researchers from Estonian University of Life Sciences started long-term field experiments with different *Vitis vinifera* and hybrid grape cultivars. At the same time, experimental vineyards were established in different parts of Estonia. The experiments showed that *V. vinifera* cultivars are not winter hardy (cannot stand warm and cold weather periods fluctuations). Hybrid cultivars 'Hasansky Sladky', 'Zilga', and 'Rondo' were found to be perspective cultivars. Hence in 2007, field trials with those cultivars in the university vineyard began. Hybrid vines can be cultivated environmentally friendly, because these cultivars are more resistant to diseases. However, data on hybrid cultivars berry quality remained insufficient. To ensure viticulture sustainability in Nordic countries, further research was needed.

In 2009, the first Estonian official wine was produced from 'Rondo' cultivar grown at Jaagu Annemäe farm vineyard. The first experimental wines of Estonian University of Life Sciences were produced from 'Hasansky Sladky', 'Zilga', and 'Rondo'. The results demonstrated that although polyphenolic spectra of red wines produced from these cultivars were generally similar to those of traditional wines, they showed a wider range of anthocyanins and a balanced phenolic acid profile (Pedastsaar *et al.* 2014). In the last five years, several small businesses started wine production. In 2020, the producers organised the Estonian Wine Route Tour, which included 18 producers, of which 7 make wines from local grapes (Estonian Wine Route Tour 2020). Red, rose, white and sparkling wines are produced and sold. Järiste winery red wine from 'Regent' and Murimäe winery red wine from 'Zilga' have received awards at international competitions.

In Estonia, a „List of recommended cultivars in Estonia" has been compiled by the Estonian Horticultural Association. Since 2010, vine cultivars are also added to the list. In 2013 the list contains 4 table and wine cultivars for vineyards (Soovitussortiment 2013). 'Hasansky Sladky' and 'Zilga' were recommended for open field cultivation. 'Supaga' and 'Aljošenkin' were recommended for tunnel cultivation. The quality of grapes and the impact of cultivation technologies needed further research.

Vine growth and ripening of yield can be influenced with viticultural practices. The novelty of this thesis is that vintage (harvest year in viticulture) dependent technological and phenolic maturity parameters were identified during long-term monitoring. Furthermore, acids compositions needed to be studied due to reoccurring problems related to their high content in grapes. Grape skin anthocyanins profile is important for red wines, but there were no such data about hybrid cultivars in Estonia. All previously mentioned data are important for vine growers and wine producers to determine vinification technology.

2. VITICULTURE

2.1. Climate

Success of viticulture depends on the climate of the growing area. In viticulture, Heliothermal Index (HI) of Huglin (1978) provides information about the heliothermal potential. It ranges from ≤ 1500 ºC (very cool) to > 3000 ºC (very warm) (Tonietto and Carbonneau 2004). HI has been used along with other temperature indices to define a region's potential for viticulture (Jones *et al.* 2010). This provides a parameter describing the suitability of a region for cultivating different grapevine cultivars based on daily mean and maximum temperatures, and on a factor denoted as the length of day coefficient, which depends on the geographic latitude of the region (and thus the average day length during the growing season).

In Europe, a traditional viticulture region lies between 30° and 50° N (Gustafsson and Mårtensson 2005). However, in spite of the harsh climate, grapevines are cultivated and grapes are produced above 50° N. Miidla (1964) pointed out that vine is resilient and adaptable if propagated vegetatively in local climate. Also, metabolic processes of the vine are normal or even more intense in the north than in the south, which compensates for the smaller amount of heat. According to the monitoring over ten years in southernmost Finland, grape cultivation under existing weather conditions is possible (Karvonen 2014a). In Tuusula area, HI was 916 in 2015, and therefore, this area belongs to very cool viticultural area (Karvonen 2017). Nevertheless, it is possible to cultivate early cultivars there.

In traditional viticulture regions, the growing season is becoming warmer (Jones *et al.* 2005). Global warming is also observed in colder climate regions, such as Estonia. Since the second half of the 20th century, the temperature rise in Estonia is faster than the global average (Fig 1) (Luhamaa *et al.* 2015). According to Luhamaa *et al.* (2014) climate scenarios for 2040-2070, winters become warmer by 2.3 to 3.1 °C and springs by 2.4 to 3.4 °C. Summer and autumn temperatures are rising by about 1.6 to 2.2 °C. The sum of active temperatures (SAT) would rise by about 250 °C per vegetation period. The models project the increasing amounts of extreme rainfalls and the elevated probability of their occurrence in summer by 124 to 165%. The models predict significant decrease of the snow cover by the end of 21st century (Luhamaa *et al.* 2015).

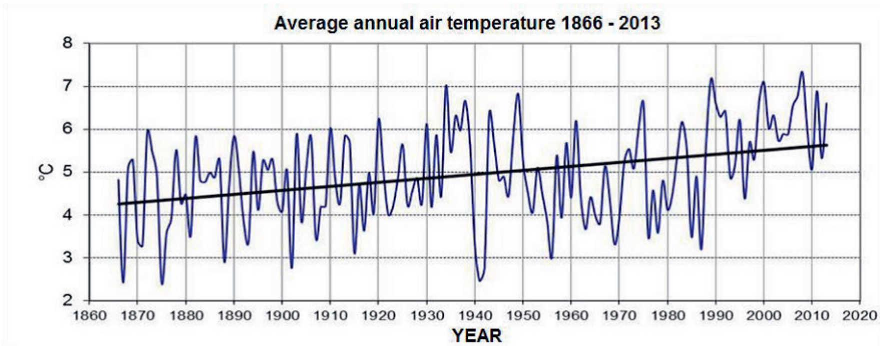


Figure 1. Annual average air temperatures in Tartu-Tõravere weather station, South-Estonia (Luhamaa *et al.* 2015).

In colder climate conditions, vine growers face several challenges: late spring and early autumn frosts, occasionally cool summers, severe winters, and short growing seasons (Gustafsson and Mårtensson 2005). Estonia faces several climate risks that lead to plant damage and decline in fruit quality. However, for viticulture there are more climate opportunities than risks (Sustainable adaptation … 2017). According to the results of the work carried out under the project AgriAdapt, the temperature rise in Estonia has a positive impact on increasing grapes soluble solids (SSC) and decreasing organic acids content (TAC) to recommended level and consequent wine quality enhancement. Longer vegetation period promotes cultivation of cultivars adapted to a warmer climate and cultivating table grapes outdoors. However, highly fluctuating winter temperature and precipitation cause plants to break dormancy and get susceptible to cold damage. In spring, shoots start to develop earlier and they are susceptible to late frost damage. Due to warmer winters, higher pest and diseases pressure emerging cause direct and indirect yield loss. Increase in extreme weather conditions in summer like hail, drought and excess water may lead to loss of grape quality.

In viticulture and vinification, work outcome depends on different factors. In wine grape cultivation, the term *terroir* brings all factors together (Deloire *et al.* 2005, Tarricone *et al.* 2013, Edo-Roca *et al.* 2013). French winemakers, who noticed differences in wines from different regions and vineyards, developed the concept of *terroir*. It involves interactions between a cultivar, climate (macro-, meso-, topo- and microclimate), soil conditions (geology, pedology), vine water status, and human factors (viticultural practices) (Van Leeuwen and Seguin 2006, Anesi 2015). According to Pereira *et al.* (2006), grape metabolic profile is more affected by climatic characteristics (temperature and water balance) than soil conditions. The extent and timing of water stress is equally important (Hunter and Deloire 2006). Based on this, grapes cultivated in Estonia and the wine made from them are unique and specific to this region; however, they still differ from region to region within the country (Karp and Lajal 2016). Therefore, experiments aimed to help Estonian vine growers and wine producers find suitable cultivars for local climate and emphasize the specificity of Estonian wine through the *terroir* aspect (cultivation technologies, cultivar selection, growing environment and climate) are needed.

In the middle of 20th century in Estonia, mainly *V. vinifera* cultivars were cultivated in greenhouses (Miidla 1964). Nowadays, vines are cultivated in field conditions and in high polyethylene tunnel. Tunnel cultivation helps improve temperature conditions. Moreover, high tunnel production allows earlier yield (Kamiloğlu *et al.* 2011). It could prolong growing season, stimulate earlier vine bud break, and decrease vine injuries due to spring and autumn frosts. In Estonia, table grapes for commercial consumption are still mainly cultivated in tunnels.

Cultivar selection is important and thus, *V. vinifera* and *V. labrusca*, *V. riparia*, *V. rupestris*, *V. aestivalis* (Lisek 2012), and *V. amurensis* (Gustafsson and Mårtensson 2005) hybrids are suitable for cultivating in cool climate. In Finland, 'Zilga' and 'Rondo' have been tested in the field (Karvonen 2014a, Karvonen 2015) and have revealed potential as desired quality wine grapes. In Estonia, different techniques have been tested in field conditions. For instance, pruning time can affect the resistance to spring damage (Rätsep *et al.* 2014). In case of autumn pruning, the emergence of buds in the spring is earlier and the risk of night frost damage is higher. In case of spring cutting, the onset of growth is postponed, and therefore, the risk of damage is lower.

2.2. Technological maturity

Technological maturity of grapes is based on pulp sugar and acids contents and pH value. In very cool climate conditions, there is a problem with reaching the desired maturity; more precisely, there is a problem with low sugar and high TAC (Gustafsson and Mårtensson 2005). Grapes are not harvested until mature, because they do not ripen after harvest (Nelson 1985). For picking time selection, growers mainly use SSC as an indicator of ripeness (Muñoz-Robredo *et al*. 2011), and it depends on the cultivar and cultivation area (Nelson 1985).

There are marketing standards for table grapes. Most common parameter inspected before sale is SSC, and its value depends on the cultivar. Table grapes can have lower SSC at harvest than wine grapes (Liu *et al*. 2006). In the European Union, minimum SSC levels are given as 12 °Brix for the cultivars 'Alphonse Lavalleé', 'Cardinal', and 'Victoria', 13 °Brix – for all other seeded cultivars, and 14 °Brix – for all seedless cultivars (OJ L-157 15/06/2011). These standards are the same in Afghanistan, but the minimum °Brix for the Indian market is 16 (ETN 300 2004). According to the International Organisation of Vine and Wine (OIV, 2008), table grapes with a Brix degree equal to or above 16 are considered suitable for harvest. In the United States, in California and other early production areas, the minimum SSC is 16.5 °Brix (Chervin *et al*. 2012). However, it is important to measure TAC and its ratio to SSC. Jayasena and Cameron (2008) found that SSC/TAC was highly correlated with consumer acceptability of 'Crimson Seedless' table grapes. Consumer acceptance increased from 33 to 87% with the increase in SSC/TAC from 20 to 40.

For winemakers, SSC is the most practical parameter to measure because sugar concentration determines wine potential alcohol content (Liu *et al*. 2006, Nogales-Bueno *et al*. 2014) and its sensory attributes (Heymann *et al*. 2013). In the experiment with 'Cabernet Sauvignon', wines made from grapes with lower sugar content turned sourer and had more fresh vegetative flavour, while those made from grapes with high SSC had stronger and bitterer taste, and in some cases, had more intensive fruit flavour and sweetness (Heymann *et al*. 2013). Recommended SSC in grapes used for red wine production ranges from 20 to 23 °Brix and for white wines – from 19.5 to 23 °Brix (Van Schalkwyk and Archer 2000). *Labrusca* type hybrid grapes can be harvested earlier (17 to 18 °Brix) before intensive *labrusca* flavour develops (Plocher and Parke 2008). In southernmost Finland, 'Zilga' SSC was on average 19 °Brix at harvest (Karvonen 2014b).

The recommended TAC and pH values should range respectively from 6.5 to 7.5 g L^{-1} and 3.2 to 3.4 for red wines, and from 7 to 8 g L^{-1} and 3.0 to 3.3 for white wines (Van Schalkwyk and Archer 2000). TAC and pH affect wine quality, shelf-life, colour, and taste. According to Tarara *et al.* (2008), higher temperatures during ripening decrease acids content and increase juice pH.

Grapes technological maturity depends on canopy management techniques such as pre-*veraison* suckering removal, shoot positioning, topping, leaf thinning and leaf removal adjacent to berry clusters at the beginning of *veraison* (Plocher and Parke 2008), cane-girdling and cluster-berry thinning (Keskin *et al.* 2013). Leaf thinning at pea-size fruit growth stage increased glucose and fructose concentration and decreased malic acid (MA) concentration in grapes (Hunter *et al.* 2004). Leaf removal at *veraison* generally increased sugar and decreased TAC (Baiano *et al.* 2015). In Estonia, different defoliation techniques have been tested to regulate grape ripening (Maante *et al.* 2016). SSC was higher and TAC showed the lowest content when leaves were removed at the beginning of *veraison*. The grapes were sweeter and pH higher when leaves were removed from both sides of the cluster.

Canopy pruning time affects budburst (Martin and Dunn 2000). Late pruning delayed phenology, and changed fruit composition of 'Pinot Noir' grapevines (Gatti *et al.* 2019). Late pruning postponed technological maturity – increased TAC and reduced SSC. In Estonia, spur and cane pruning methods at different dormancy phases (eco- and endodormancy) have been tested (Rätsep *et al.* 2014). Autumn spur pruning increased SSC and autumn cane pruning decreased TAC. Pruning in spring significantly decreased SSC/TAC for both pruning methods. However, technological maturity proved to be a problem because grapes still had high acid and low sugar content.

Hybrid grape cultivars such as 'Hasansky Sladky', 'Zilga', and 'Rondo' are common for Estonian vine growers, but scientific knowledge on the proportion of individual acids in such cultivars is insufficient for vinification. Tartaric acid (TA) and MA are the most abundant organic acids in grapes and generally account for 62 to 92% of all organic acids (Kliewer 1966). Higher MA content causes a sour/green/sharp taste in wine (Volschenk *et al*. 2006). A high ratio of TA to MA improves wine stability (Liu *et al*. 2007).

The acids are synthesised actively up to *veraison*, after which TA content stays relatively constant (Ruffner 1982, Volschenk *et al*. 2006). MA content starts to decrease due to its metabolism through different pathways (Sweetman *et al*. 2009). MA degradation rate depends on the temperature of growing environment: higher temperatures at *veraison* and during ripening stages reduce MA content (Sweetman *et al*. 2014, Kizildeniz *et al*. 2018). MA level is usually higher in cool climate regions and lower in warmer regions (Volschenk *et al*. 2006, Conde *et al*. 2007). TA is not as rapidly lost at high temperatures as MA (Kliewer 1971). Also, leaf thinning at pea-size fruit development stage reduced MA concentration and influenced tartaric and malic acids ratio (TA/MA) (Hunter *et al*. 2004). Based on the previously mentioned aspects, more precise information on TA and MA contents and their ratio in wine grapes will lead to the understanding of necessity of malolactic fermentation and selection of appropriate yeast strains for vinification process.

2.3. Phenolic maturity

Phenolic maturity depends on content of phenolics in grape skin. Polyphenols are important for red wine colour intensity, astringency and bitterness. Grape phenolic content and profile depend on the cultivar, growing area, weather conditions and viticultural practices (Katalinić *et al*. 2010, Tarko *et al*. 2010, Zhu *et al*. 2012, Soubeyrand *et al*. 2014, Samoticha *et al*. 2017, Fernandes De Oliveira *et al*. 2017, Wojdyło *et al*. 2018). Cultivars with a higher total phenolic content (TPC) tend to have higher total antioxidant activity (Yang *et al*. 2009, Liang *et al*. 2014). Wine pH is important because changes in its level can cause reversible structural transformations in anthocyanins molecules, which can have an effect on their colour (He *et al*. 2012).

So far, phenolic compounds of cultivars 'Hasansky Sladky' and 'Zilga' were significantly affected by viticultural practice such as defoliation (Maante *et al*. 2016). TPC showed the highest content in both cultivars when leaves were removed from one side of the cluster at the beginning of *veraison*. Total anthocyanin content (ACC) of grapes was more dependent on the cultivar and weather than on defoliation treatment. Other affecting techniques were pruning methods (Rätsep *et al.* 2014). In the two years' mean, spring pruning decreased TPC up to 22% in spur and cane pruning treatments. Spring cane pruning increased ACC. There were no long-term data about weather impact on phenolic composition of hybrid cultivars 'Hasansky Sladky', 'Zilga', and 'Rondo'. TPC and ACC varied significantly in wines produced from these cultivars (Pedastsaar *et al.* 2014).

Anthocyanins are the main compounds responsible for the red colour of grapes and they are synthesised to protect the skin from the negative effect of the environment, especially UV radiation. Colour is an important factor for evaluating red wine quality and is one of the most important factors for consumers when choosing a wine. Anthocyanin composition depends on the genetic background of *Vitis* species (Liang *et al.* 2008). However, it is also affected by climate (Ortega-Regules *et al.* 2006) and different viticultural practices (Downey *et al.* 2004, Basile *et al.* 2018). Elevated temperatures during ripening may reduce the accumulation of anthocyanins and could partly degrade the previously synthesized components (Mori *et al.* 2007, Poudel *et al.* 2009, Cheng *et al.* 2014). Therefore, in hot regions, anthocyanin accumulation is inhibited in the skins of red and black grapes, but cooler conditions are more favourable.

In red wines, grape skin anthocyanins not only provide appealing colour but also play an important role in wine organoleptic quality. The colour of anthocyanidins is different and the visible colour of entire molecule shift from orange to violet (Fig 2) (Ananga *et al.* 2013). Various reports have referred to the relationship between wine colour and anthocyanin composition. Delphinidin-3-O-glucoside (Dp) contributed most to the colour of 'Cabernet Sauvignon' and 'Merlot' wine, but malvidin-3-O-glucoside (Mv) – 'Cabernet Gernischt' wine (Tang *et al.* 2017). In wines produced from hybrid cultivars in Estonia, Mv was the most abundant, accounting for 58 to 62% of the pigments (Pedastsaar *et al.* 2014). Genetic background significantly affects the content and profile of anthocyanins. Therefore, individual anthocyanin profile has been used as a tool to assess the varietal origin of single cultivar wines, being called the "anthocyanin fingerprint" (Revilla *et al.* 2016). Hybrids differ in their phenolic and anthocyanin profiles from *V. vinifera* cultivars (Zhu *et al.* 2012, Samoticha *et al.* 2017, Wojdyło *et al.* 2018).

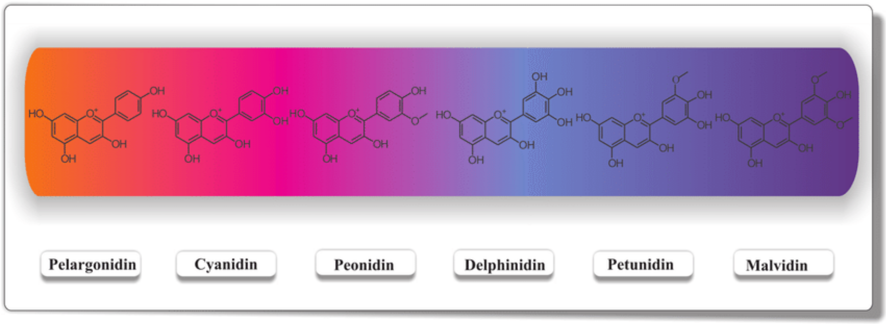


Figure 2. Visible colour range of common anthocyanidins (Ananga *et al.* 2013).

3. HYPOTHESES AND AIMS OF THE STUDY

The biochemical composition of table and wine grapes is affected by the cultivar properties, vintage and viticultural practices. Most of the research has been done with different *V. vinifera* cultivars. There is a little information about interspecific hybrid grapevine cultivars in cool climate conditions. The extent of variability of maturity parameters in cool climate is also less known. Based on previous research and experience, the following hypotheses were set:

- In Estonia, it is possible to cultivate table grapes with required marketing standards.

- The content of primary and secondary metabolites of interspecific hybrid grapevines depends significantly on the Estonian weather, cultivar properties and viticultural practices. However, it is not known how significant the influence is and whether the recommended maturity levels can be reached.

- Cultivating vines in a polyethylene tunnel has a positive effect on technological and phenolic maturity parameters of wine grapes.

The aims of the present study were to determine the effect of:

- cultivar properties on SSC **(I)**, TAC and their ratio in table grapes;

- vintage on technological and phenolic maturity of hybrid grapes 'Hasansky Sladky', 'Zilga', and 'Rondo' **(III, IV)**;

- viticultural practices on fruit technological and phenolic maturity parameters of hybrid grapes 'Hasansky Sladky', 'Zilga', 'Rondo', and 'Supaga' **(II, III, IV)**.

4. MATERIAL AND METHODS

4.1. Experimental sites and maintenance

Vine experiments were conducted from 2009 to 2018 in field and from 2013 to 2018 in high polyethylene tunnel (Table 1, Fig 3, Table 2):

- **Site 1.** Vineyard at Estonian University of Life Sciences experimental station in Tartu County (58° 21' N; 26° 31' E), with own-rooted plants of hybrid grape cultivars: 'Hasansky Sladky', 'Zilga', 'Rondo', and 'Supaga'. Vines were planted in 2 × 2 m spaces, trained on low double trunk trellis with 12 buds left per plant. The experimental design was a randomised block with 4 replicates and 8 vines in each (**II-IV**).

- **Site 2.** High polyethylene tunnel in Tartu County (58 ° 17' N; 26° 33' E) was 28 m long, 7.6 m wide and 4.6 m high, covered with 0.18 mm thick UV stable low-density polyethylene. Own-rooted 'Rondo' plants were planted in 1.6 × 2 m spaces, trained on low double trunk trellis and with 12 buds left per plant. White polypropylene fabric was used as a winter cover. The experimental design was a randomized block with 3 replicates and 3 vines in each (**III-IV**).

- **Site 3.** High polyethylene tunnel in Pärnu County (58° 37' N; 25° 8' E) was 45 m long, 8 m wide and 4 m high, covered with 0.18 thick UV stable low density polyethylene. Experimental cultivars were own-rooted plants of 'Osella', 'Kosmonavt', 'Mars', 'Swenson Red', 'Somerset Seedless', 'Canadice', 'Arkadia', 'Supaga', 'Hasansky Sladky', and 'Zilga'. Vines were planted in 1.65 × 3.5 m spaces. Vines training system was high double trunk trellis. White polypropylene fabric and spruce (*Picea*) branches were used as vine winter cover. The experimental design was a randomized block with 3 replicates and 3 vines in each (**I-II**).

Table 1. Overview of the conducted experiments

Paper	Cultivars	Impact factor	Analysed parameters	Year
I	'Kosmonavt', 'Swenson Red', 'Somerset Seedless', 'Mars', 'Canadice', 'Osella', 'Arkadia', 'Supaga'	Cultivar	SSC, TAC_{titr}, SSC/TAC	2013-2014
II	'Hasansky Sladky', 'Zilga', 'Supaga'	Viticultural practice	SSC, TAC_{titr}, SSC/TAC, pH, TPC, ACC_{spec}	2013-2015
III	'Hasansky Sladky', 'Zilga', 'Rondo'	Vintage, viticultural practice	HI, SSC, TAC_{titr}, TAC_{spec}, TA, MA, pH, TA/MA	2009-2018
IV	'Hasansky Sladky', 'Zilga', 'Rondo'	Vintage, viticultural practice	HI, ACC_{spec}, ACC_{HPLC}, TPC, Cy, Dp, Pn, Pt, Mv, NBC, CW, BW	2010-2018

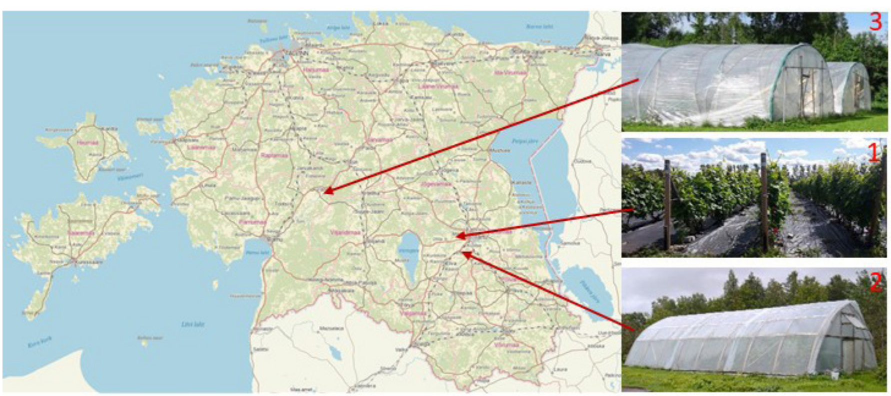


Figure 3. Locations of experimental sites. 1 – Tartu County vineyard; 2 – Tartu County tunnel; 3 – Pärnu County tunnel (Photos: M. Maante-Kuljus, K. Karp)

Table 2. Experimental cultivars (Photos: M. Maante-Kuljus, E. Vool)

'Osella' (table grape)	**'Kosmonavt' (table grape)**	**'Mars' (table grape)**
		
'Swenson Red' (table grape)	**'Somerset Seedless' (table grape)**	**'Canadice' (table grape)**
		
'Supaga' (wine grape)	**'Arkadia' (table grape)**	**'Hasansky Sladky' (wine grape)**
		
'Zilga' (wine and table grape)	**'Rondo' (wine grape)**	
		

In all experimental vineyards, the ground was covered with black synthetic mulch, and no irrigation system was used. No additional mineral fertilizers were used at any experimental area, except for site 3 where compost was used in the planting holes during planting. Branches were pruned after sap flow ended in spring. After inflorescence formation, shoots were thinned (fruitless shoots were removed). Every two weeks, lateral shoots and main shoots were cut back. Shoots height was about eight leaves after clusters. Leaves from the cluster zone were removed at the beginning of *veraison*. During *veraison*, SSC was determined once a week on randomly chosen grapes from basal clusters. Harvest time was determined when SSC did not change significantly or due to the weather conditions (arrival of night frosts).

4.2. Measurements and analysis

Weather conditions. Weather data were obtained from Estonian Environment Agency Tartu-Tõravere and Kaansoo meteorological stations. Air temperature (°C), precipitation (Prec, mm), and relative air humidity (RH, %) were recorded in the observation area every full hour 24 hours a day. Air temperature in tunnels was recorded with temperature data loggers every full hour 24 hours a day. SAT was calculated by summing daily average temperatures above 10 °C (month, year). Throughout grape development (Phenologigal growth stage identification scale – BBCH 71-79, June-July) and *veraison* (BBCH 81-89, August-September), SAT was calculated. Radiation flux (RF, W m^2) data were obtained from Tartu University Laboratory of Environmental Physics. RF was recorded in the observation area every five minutes 24 hours a day.

HI was calculated using the following expression (Huglin 1978) (**III-IV**):

$$HI = \sum_{Mi}^{Mf} \left[\frac{(T - 10) + (T_{max} - 10)}{2} \right] \times d$$

where ‘T’ and ‘Tmax’ are, the average mean and maximum monthly temperature (°C), respectively; ‘Mi’ and ‘Mf’ are the initial and the final month of the period, respectively; ‘d’ is the length of day coefficient, with value of 1.09 for latitudes 58°.

Grape cluster parameters. BBCH scale was used in phenological observations (Lorenz *et al.* 1994). Phenological observations were made once a week over the vegetation period (April to October). Yield analyses were made on the basal cluster of each plant. Berries per cluster (NBC) were recorded and berry weight (BW) was calculated as the mean of 100 berries (**IV**). Cluster weight (CW) of ten randomly selected grape bunches of a vine was determined in each replication.

Technological maturity parameters. At harvest, SSC (°Brix) of berries was determined using a digital refractometer (Atago Pocket Refractometer Pal-1) (**II**, **III**). SSC accumulation measurements were conducted from 8 August to 20 September 2013 and from 7 August to 12 September 2014 (**I**). 30 grapes from each of the three replications from different parts of clusters were analysed.

Subsequent analyses were performed on previously frozen and then thawed berries. Collected fruit samples were stored at −20 ºC until the analysis (**II-IV**). 400 g of grapes from each of the three replications from the different parts of the basal cluster were analysed. From one replication three separate extractions were made.

Organic acids content titrimetrically (TAC_{titr}) was determined in juice by the titration with 0.1 M NaOH solution to the endpoint of pH of 8.2 (Wrolstad *et al.* 2005), using a Mettler Toledo EasyPlus Titration titrator (with electrode DG 111-SC for endpoint detections) (**II-IV**). TAC_{titr} was expressed as g of tartaric acid per L (**III**) or g per 100 g (**II**, Fig 6) of juice. Soluble solids and organic acids ratio (SSC/TAC_{titr}) was calculated (Fig 6). Grape juice pH was measured with pH/conductivity-meter (HD 2156.1, Delta OHM) (**II-IV**). TA, MA, and TAC_{spec} contents were estimated by FT-IR spectroscopy (Edelmann *et al.* 2003) and the measurements were performed using FT-IR Wine & Must Analyzer (ALPHA, Bruker Optics, Ettlingen, Germany) (**III**). TAC_{spec} was expressed as g per L of juice. The ratio between TA and MA was calculated.

Phenolic maturity parameters. Phenolic maturity parameters were determined from grape skin. TPC was determined by the Folin-Ciocalteu phenol reagent method (Wrolstad *et al.* 2005) with a spectrophotometer (UVmini-1240 Shimadzu, Kyoto, Japan) at 765 nm (**II, IV**). Ethanol-acetone (7:3) solution was used as a solvent to extract total phenolic compounds (5 g of berries skins were added to 50 mL of solution). TPC was expressed as mg of gallic acid equivalent per 100 g of FW (**II, IV**).

Total anthocyanins content spectrophotometrically (ACC_{spec}) of grape skin was determined applying pH-differential method (Wrolstad *et al.* 2005) (**II, IV**). Hydrochloric acid-ethanol (15:85) solution was used as a solvent to extract ACC_{spec} (10 g of berry skins were added to 100 mL of solution). Absorbance was measured with a spectrophotometer (Uvmini-1240 Shimadzu, Kyoto, Japan) at 510 and 700 nm, in buffers with pH levels of 1.0 (HCl 0.1N) and 4.5 (citrate buffer). ACC_{spec} was expressed as mg of malvidin-3-glucoside equivalent per 100 g of FW.

Total anthocyanins content chromatographically (ACC_{HPLC}) and individual anthocyanins (Fig 4) were determined using the method for polyphenol profiling (Lambert *et al.* 2015) with some modifications (**IV**). Briefly, the samples were prepared in three replicates; approx. 1 g of berry skin sample was added to 50% ethanol + 1% HCl (v:v) solution. Qualitative and quantitative analyses were performed on a Shimadzu Nexera X2 UHPLC-DAD system coupled to triple quadrupole mass spectrometer LCMS 8040 (Shimadzu Scientific Instruments, Kyoto, Japan). Chromatographic separation of anthocyanins was implemented on a reverse phase column ACE Excel 3 C18- PFP (100 × 2.1 mm × 3μm; from ACE® Advanced Chromatography Technologies Ltd., Aberdeen, Scotland) with pre-column (SecurityGuard ULTRA, C18; from Phenomenex, Torrance, CA, USA). Individual anthocyanins were identified comparing retention times, UV spectra, as well as parent and daughter ion masses with those of standard compounds and literature data. Anthocyanins were quantified at the wavelength of 520 nm and results of ACC_{HPLC} expressed as mg of malvidin-3-O-glucoside equivalent per 100 g of FW. Major monoanthocyanidins detected were Dp, cyanidin-3-O-glucoside (Cy), petunidin-3-O-glucoside (Pt), peonidin-3-O-glucoside (Pn), and Mv.

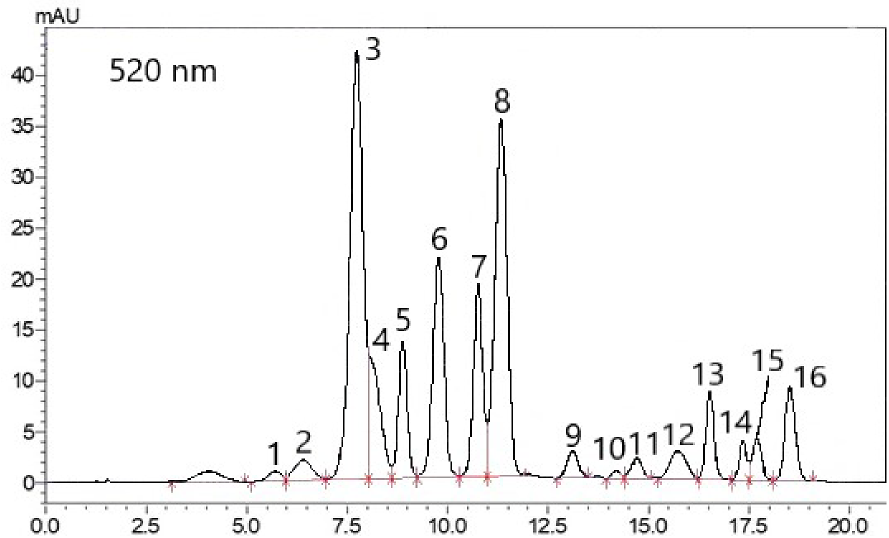


Figure 4. An example of anthocyanins profile of 'Rondo': (1) cyanidin-3-O-glucoside-5-O-glucoside; (2) petunidin-3-O-glucoside-5-O-glucoside; (3) De; (4) malvidin-3-O-glucoside-5-O-glucoside; (5) Cy; (6) Pt; (7) Pn; (8) - Mv; (9) delphinidin-3-O-(6"-O-acetyl)-glucoside; (10) cyanidin-3-O-(6-O-acetyl)-glucoside; (11) petunidin-3-O-(6"-O-acetyl)-glucoside; (12) malvidin-3-O-(6-O-acetyl)-glucoside; (13) delphinidin-3-O-(6"-p-coumaroyl)-glucoside; (14) cyanidin-3-O-(6"-p-coumaroyl)-glucoside; (15) petunidin-3-O-(6"-O-coumaroyl)-glucoside; (16) peonidin-3-O-(6"-O-coumaroyl)-glucoside.

4.3. Soil and weather conditions

Soil conditions. Site 1 soil was high-productivity sandy loam *Haplic Luvisol* with sufficient drainage (Table 3). The contents of available P and K were determined by the ammonium lactate method (Egnér *et al.* 1960).

Site 2 soil was high-productivity sandy loam *Haplic Luvisol* with sufficient drainage (Table 3). Site 3 soil was loam sandy *Gleyic Albeluvisol*. This soil was with prolonged surface and perched water. A road drainage ditch was located near the tunnel.

Table 3. Soil condition in experimental sites

Site	Soil	Soil type	pH_{KCl}	P	K	Mg	Ca
				mg kg^{-1}			
Site 1	*Haplic Luvisol*	Sandy loam	5.4	147	257	260	1670
Site 2	*Haplic Luvisol*	Sandy loam	5.4	159	574	578	2381
Site 3	*Gleyic Albeluvisol*	Loam sandy	5.6	22	170	98	830

Weather conditions. Site 1 and 2. In most experimental years, the warmest month was July, except in 2015 and 2017, when it was warmer in August. In spring months, mean temperature in May was 12.5 °C and in June – 15.2 °C. At *veraison*, mean temperature in July was 18.5 °C, in August – 16.7 °C, and in September – 12.2 °C. Mean monthly temperatures in 2018 were higher every month compared to the mean of experimental years. Temperatures in tunnel conditions were higher 0.6 to 5.9 °C. The last spring frost occurred usually at the beginning of May, except in 2009 and 2017, when it was in mid-May. The average length of the frost-free period (FFP) was 158 days and ranged from 140 to 180 days. The first autumn frost was mostly in the second half of October, but in four years, it was at the end of September or at the beginning of October.

The 10 year-average Prec from April to October was 462 mm. The amount of Prec ranged from 325 to 566 mm. The driest year was 2011, when Prec from April to October was 325 mm, and the wettest year was 2010 (566 mm). RH ranged from 53 to 91%. The lowest RH months were April and May, when the average monthly RH was 68% and 65% respectively. The highest RH months were September and October, when it was 82% and 84% respectively. RF for the years from 2016 to 2018 was 153 to 183 W m^2 in August and 93 to 120 W m^2 in September.

SAT (≥10 °C) for the years from 2009 to 2018 ranged from 1981 to 2660 °C and average was 2304 °C (Table 3, **III**). In half of the experimental years, SAT exceeded the mean of 10 years. The warmest months were July and August, when the average monthly SAT was 578 °C and 522 °C, respectively. The average SAT for vine growth period BBCH 71-79 was 1031 °C and ranged from 877 to 1167 °C. For growth period BBCH 81-89, SAT ranged from 789 to 938 °C and average was 851 °C. The warmest spring was in 2013, 2016, and 2018. In August, SAT was high in 2010, 2013, 2014, 2015, and 2018, but in September – in 2009, 2011, 2014, and 2018.

Site 3. June was the warmest month in 2013, while in 2014 and 2015, it was July. Temperature fluctuation was greater between daily minimum and maximum temperatures in spring months (May, June), in 2013 and 2015 (the difference was from 10.0 to 19.1 °C). FFP was 136 days in 2013 and 2014, and 163 days – in 2015. The last spring frost was on 28 April, 1 May, and 25 April in 2013, 2014, and 2015, respectively. In two experimental years, the first autumn frost was recorded in the tunnel earlier than in the field. The amount of Prec from May to September in tunnel area ranged from 259 to 324 mm. The most arid year was 2013 and the wettest year – 2014.

4.4. Statistical analysis

The results of SSC **(I)**, TAC_{titr}, and SSC/TAC_{titr} (Fig 6) of table grape cultivars were tested by one-way ANOVA (Fig 5). To evaluate the effect of cultivars, $LSD_{0.05}$ was calculated. Different letters on figures mark significant differences at $p \leq 0.05$.

In field experiment with wine grapes, the results of SSC, TAC_{titr} and pH (in juice), ACC_{spec}, TPC, and monoanthocyanidins (in skin) were tested by one-way ANOVA **(III, IV)** (Fig 5). To evaluate the effect of vintage, $LSD_{0.05}$ was calculated, and the different letters in tables mark a significant difference at $p \leq 0.05$. To evaluate main effect of cultivar and vintage, two-way analysis of ANOVA was carried out, and the difference was marked as non-significant (ns) or, using confidence levels, as significant at $p \leq 0.05$*, 0.01** or 0.001***.

Linear correlation coefficients were calculated between the variables of berry and weather parameters with coefficient significance being $p \leq 0.05$*, and 0.01**. Relationship strength was estimated as $0.3 \leq r \leq 0.7$ (moderate), and $r \geq 0.7$ (strong). With skin parameters, principal component analysis (PCA) was performed to describe the structure of all analysed parameters in relation to the cultivars and vintage **(IV)**.

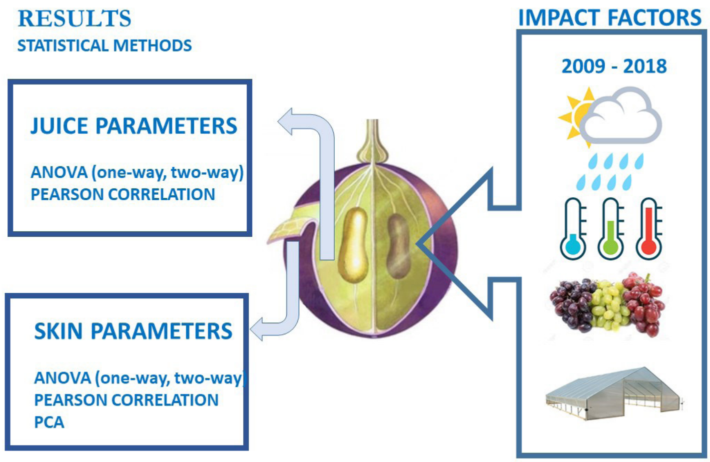


Figure 5. Statistical analysis and impact factors.

In experiment of viticultural practice, the results of 'Hasansky Sladky', 'Zilga', 'Supaga' **(II)**, and 'Rondo' **(III, IV)** were tested by one-way ANOVA (Fig 5). To evaluate the main effects of viticultural practice and vintage, two-way analysis of ANOVA was carried out and the difference was marked as non-significant (ns) or, using confidence levels, as significant at $p \leq 0.05^*$, 0.01^{**} or 0.001^{***}.

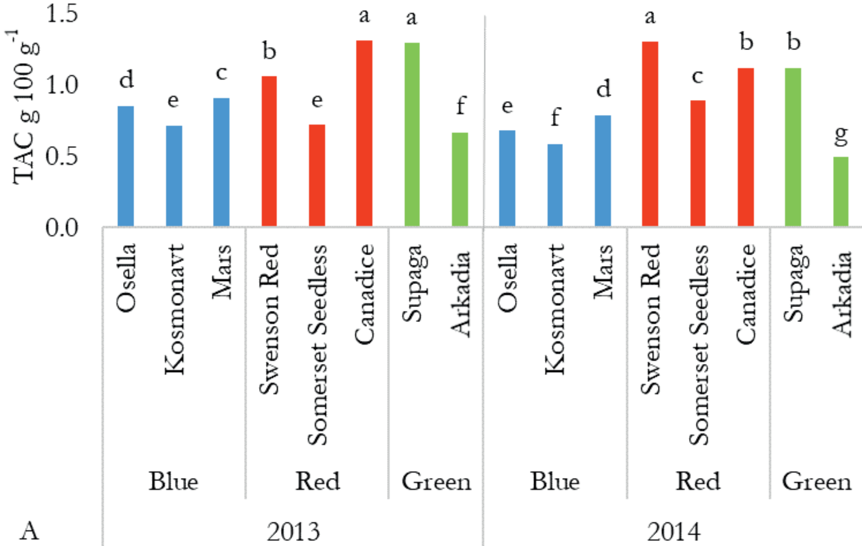
5. RESULTS

5.1. Technological maturity

Cultivar effect on table grapes in tunnel. In 2013, at harvest, SSC ranged from 15 to 23 °Brix, and in 2014 – from 14 to 18 °Brix (Fig 4, **I**). Statistically significant difference was caused by cultivar. In 2013, among blue cultivars, 'Osella' had the highest value of SSC (23 °Brix), and in both experimental years, it was significantly lower in 'Mars' (15 °Brix). In 2013, among red cultivars, 'Somerset Seedless' had the highest value of SSC (22 °Brix), but the value was the lowest in 'Swenson Red' (16 °Brix) in both experimental years. Among green cultivars, 'Supaga' had significantly higher values of SSC in both years (respectively 19 and 18 °Brix). 'Osella' and 'Kosmonavt' had shorter ripening period (Fig 2, 3, **I**). Earliest cultivars were 'Somerset Seedless', 'Kosmonavt', and 'Osella'.

TAC_{titr} ranged at the harvest from 0.67 to 1.32 g 100 g^{-1} in 2013 and 0.49 to 1.31 g 100 g^{-1} in 2014 (Fig 6A). In 2013, the highest content was found in 'Canadice' and 'Supaga', but in 2014 – in 'Swenson Red'. In both years, the lowest content was in 'Arkadia'. SSC/TAC_{titr} ranged from 14 to 31 in 2013 and from 12 to 30 in 2014 (Fig 6B). In 2013, the highest ratio was in 'Somerset Seedless', but in 2014 – in 'Kosmonavt'. The lowest ratio was in 'Canadice', 'Swenson Red', 'Supaga', and 'Mars' in 2013, but in 2014 – in 'Swenson Red'. TAC_{titr} and SSC/TAC_{titr} were significantly affected by cultivar.

Vintage and cultivar effect on wine grapes in field. In field conditions (2009-2018), SSC was significantly affected by vintage – the results ranged from 12 to 21 °Brix (Table 2, **III**). 'Hasansky Sladky' had yield every year, 'Rondo' – in eight years, and 'Zilga' – in nine years. SSC ranged from 17 to 21 °Brix in 'Hasansky Sladky', and the highest content was determined in five experimental years out of 10. For 'Rondo', SSC ranged from 12 to 17 °Brix between the years. The lowest content was found in 2012 and 2017 (13 and 12 °Brix, respectively), but the higher content in four years. For 'Zilga', results ranged between 13 and 19 °Brix, and the higher SSC was in two years and the lowest – in three years out of nine.



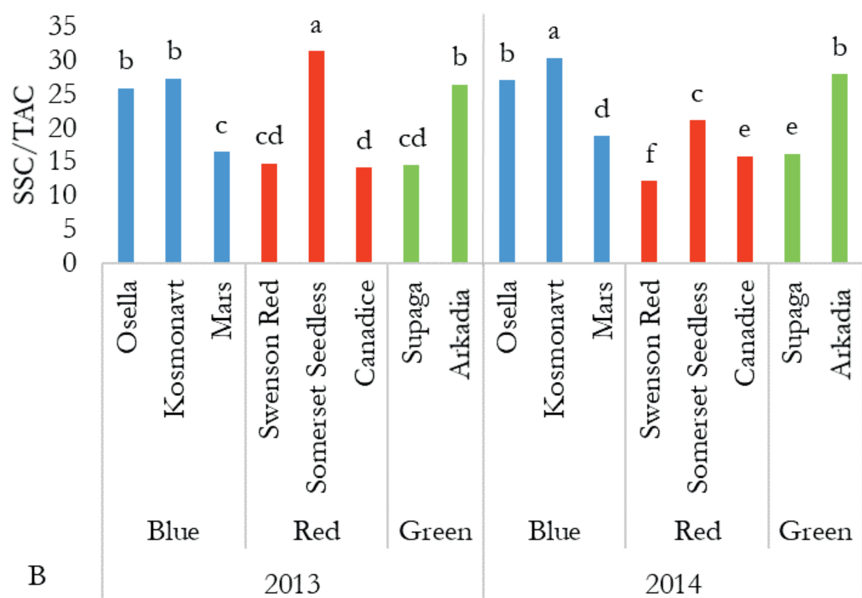


Figure 6. The effect of cultivar on TAC_{titr} (A) and SSC/TAC_{titr} (B) in tunnel in 2013 and 2014. Cultivars within the year were compared by LSD-test. Different letters on the columns mark significant differences at $p \leq 0.05$.

The impact of vintage caused a large variation in TAC_{titr} – it ranged from 6.5 to 20.7 in 'Hasansky Sladky', from 9.2 to 22.7 in 'Rondo', and from 6.7 to 17.6 g L^{-1} in 'Zilga' (Table 2, **III**). For 'Hasansky Sladky' and 'Zilga', TAC_{titr} was the lowest in 2018, for 'Rondo' – also in 2016. The highest content was in 'Hasansky Sladky' in 2012, in 'Rondo' – in 2010, and in 'Zilga' – in 2015. pH ranged between 2.8 and 3.9. The highest pH was in 'Hasansky Sladky' (3.9) in 2013, in 'Zilga' (3.5) – in 2013 and 2015, and in 'Rondo' (3.6) – in 2015. The lowest value was in 2017 in cultivars 'Hasansky Sladky' and 'Zilga', but in 'Rondo' – in 2010.

Heliothermal Index. From 1987 to 2008, HI ranged from 732 to 1422 and in experimental years – from 888 to 1532 (Fig 1, **III**). In more than a half of the test years, HI value was above the mean of 1222. In 2017, HI was the lowest and in 2018 – the highest. The trend line shows warming, but HI had large variability between test years. Experimental areas in Tartu County belong to a very cool vine cultivating zone. In 2018, when HI was above 1500 and according to this Tartu County could be classified as a cool zone.

Correlations with weather indicators. The relationships between weather indicators and technological maturity parameters showed that SSC was higher in case of warmer weather conditions in April (mean) and May (mean and SAT) and with higher HI (Table 4, **III**) (Fig 7). Temperatures of growth periods BBCH11-79 and BBCH81-89 had a positive correlation with SSC in 'Rondo' and 'Zilga', but for 'Hasansky Sladky', a positive correlation was only with BBCH11-79. SSC in 'Zilga' and 'Rondo' had a negative correlation with Prec. TAC_{titr} in 'Hasansky Sladky' and 'Zilga' had a negative correlation with SAT and HI. 'Rondo' and 'Zilga' TAC_{titr} had a negative correlation with FFP. In all cultivars, pH had a negative correlation with Prec. In 'Hasansky Sladky', pH also had a positive correlation with temperature related parameters (except FFP, which revealed no correlation). In 'Zilga', pH had a negative correlation with FFP.

Effect of viticultural practice on wine grapes. SSC of 'Supaga' was affected by viticultural practices in all experimental years - ranging from 14 (field) to 20 (tunnel) °Brix (Table 3, **II**). There were significant differences between field and tunnel cultivated fruits. In 'Hasansky Sladky' and 'Zilga', SSC was from 15 in field to 25 °Brix in tunnel and the difference was significant in two experimental years. In 'Rondo', SSC

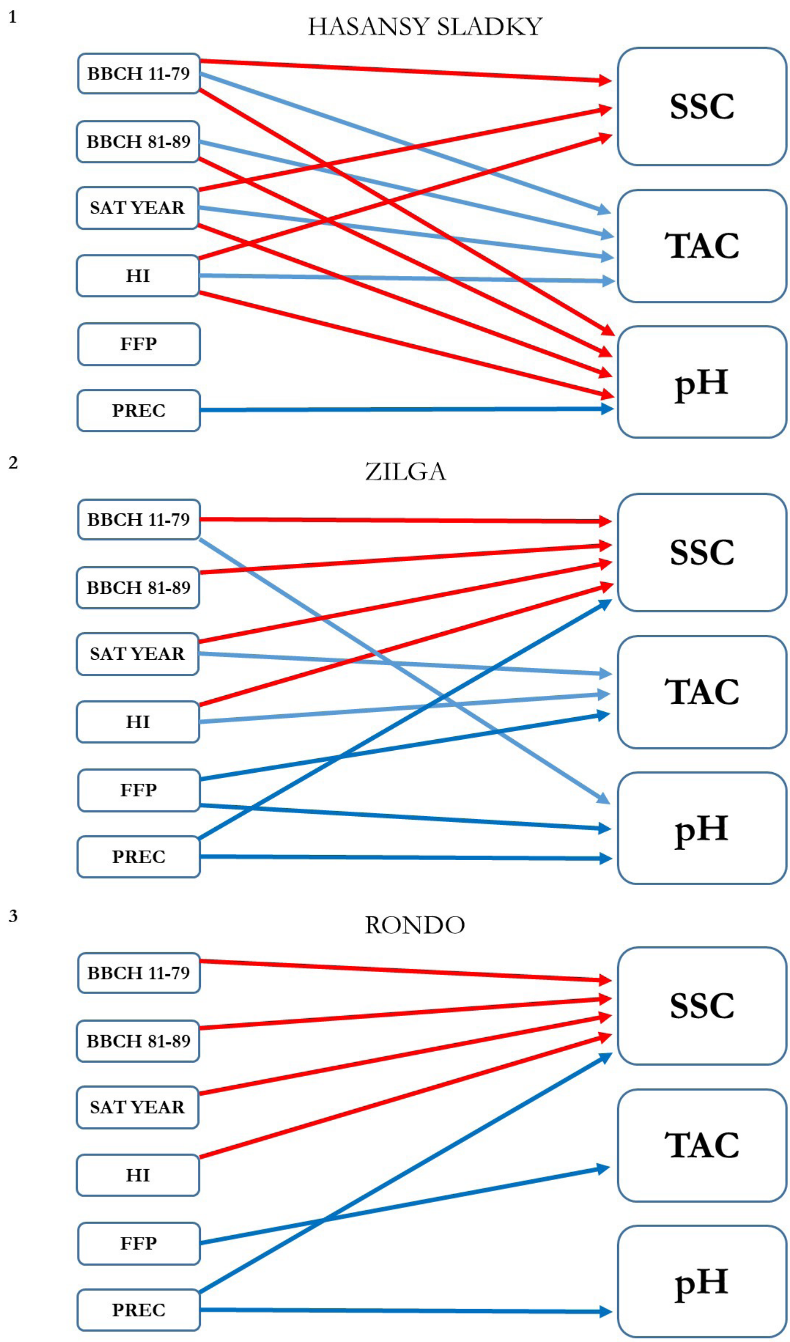


Figure 7. Correlations between technological parameters and weather data for cultivars 'Hasansky Saldky' (1), 'Zilga' (2), and 'Rondo' (3) in field conditions (2016-2018). Red colour shows positive and blue negative correlation. The significant relationships pointed out in the figure are based on the data in article III in table 4.

ranged from 15 to 18 °Brix in tunnel, and in the field – from 12 to 17 °Brix (Table 3, **III**). Viticultural practice and vintage affected the SSC in all cultivars.

TAC_{titr} in 'Zilga' and 'Supaga' varied from 1.04 to 1.76 g 100 g^{-1} and was influenced by viticultural practices (Table 3, **II**). In 'Hasansky Sladky', TAC_{titr} varied from 1.09 to 1.61 g 100 g^{-1} and viticultural practice had no effect only in 2013. In the tunnel, TAC_{titr} decrease ranged from 3 to 32%. Viticultural practice affected $SSC/TAC_{titr,}$, which varied from 8.2 to 23.3. Thus in the field, SSC/TAC_{titr} ratio decreased from 20 to 49%. For all cultivars, the main effect of viticultural practice on SSC/TAC_{titr} was significant. Growing conditions in tunnel decreased TAC_{spec} in 'Rondo' by 14%, 25%, and 34% (respectively to the years) compared to field (Table 3, **III**). For all cultivars, viticultural practice and vintage affected TAC.

Grape juice pH of 'Supaga' was significantly lower in field conditions than in the tunnel and varied over the years from 3.07 to 3.59 (Table 3, **II**). For 'Zilga' and 'Hasansky Sladky', the same effect occurred in two years. In the field, 'Zilga' pH varied between 3.08 to 3.53 and 'Hasansky Sladky' – 3.18 to 3.77. In the tunnel, 'Zilga' pH was between 3.31 to 3.60 and 'Hasansky Sladky' – 3.46 to 3.68. In the tunnel, 'Rondo' pH increased by 5.7% in 2016, but in 2017, it decreased by 8.1% (Table 3, **III**). For all cultivars, the main effect of viticultural practice and vintage was significant.

In field and tunnel experiment, 'Rondo' TA ranged from 3.6 to 5.5 g L^{-1} and the impact direction of viticultural practice differed; in 2016, tunnel cultivation increased TA by 19%, but in 2017 decreased it by 26%, and in 2018 there was no effect (Table 3, **III**). Cultivating grapes in tunnel reduced MA content by 2.3 in 2016, by 4.2 in 2017, and by 3.5 g L^{-1} in 2018. Viticultural practice caused a large variation in TA and MA ratio (from 0.7 to 2.0) which was significantly higher in the tunnel in all experimental years. TA, MA and TA/MA were significantly affected by the year (TA/MA, $p \leq 0.01$; TA and MA, $p \leq 0.001$) and viticultural practice (TA, $p \leq 0.01$; MA, and TA/MA, $p \leq 0.001$).

5.2. Phenolic maturity

Vintage and cultivar impact in field. TPC had a large variation due to vintage effect – TPC in 'Hasansky Sladky' ranged from 192 to 394 mg 100 g^{-1}, in 'Rondo' – from 374 to 671 mg 100 g^{-1}, and in 'Zilga' – from 214 to 372 mg 100 g^{-1} (Table 4, **IV**). Each cultivar had its highest content in a different vintage: 'Hasansky Sladky' – in 2011, 'Rondo' – in 2018, and 'Zilga' – in 2014 and 2016. Vintage, cultivar, and interaction between them ($p \leq 0.001$) affected TPC significantly.

Among nine harvest years, ACC_{spec} varied significantly – the contents ranged from 30 to 405 mg 100 g^{-1} (Table 4, **IV**). 'Hasansky Sladky' had a yield every year, 'Rondo' – in seven years, and 'Zilga' – in eight years. In 'Hasansky Sladky', ACC_{spec} ranged from 30 to 138 mg 100 g^{-1} and the highest content was determined in two experimental years out of nine (2010 and 2013). In 'Rondo', ACC_{spec} ranged from 75 to 405 mg 100 g^{-1} and the highest significant content was in 2018. In 'Zilga', the content ranged from 32 to 150 mg 100 g^{-1} and the highest ACC_{spec} was in one year out of eight harvest years (2018). The effect of vintage, cultivar, and interaction between them on ACC_{spec} was significant ($p \leq 0.001$).

In two years out of three, content of monoanthocyanidins was significantly higher in 'Rondo' (Table 5, **IV**). Dp and Cy contents were significantly lower in 'Hasansky Sladky' in all experimental years. Pn content did not differ significantly between 'Hasansky Sladky' and 'Zilga'. Mv and Pt content differed significantly between cultivars and years. Monoanthocyanidins were significantly affected by vintage, cultivar, and the interaction between them ($p \leq 0.001$).

Correlations with weather indicators. The correlations between weather indicators and skin maturity parameters showed in 'Zilga', 'Rondo', and 'Hasansky Sladky': a negative correlation between ACC_{HPLC} and Prec, but a positive correlation with other temperature related parameters (Table 7, **IV**) (Fig 8). ACC_{HPLC} had a positive correlation with August RF in 'Hasansky Sladky', but in 'Zilga' and 'Rondo' – with September RF. In 'Rondo', TPC had a positive correlation with temperature related parameters and September RF, but negative with Prec. In 'Hasansky Sladky', TPC had a positive correlation with August RF, but in 'Zilga' – a negative correlation. In 'Zilga', TPC had a positive correlation with Prec.

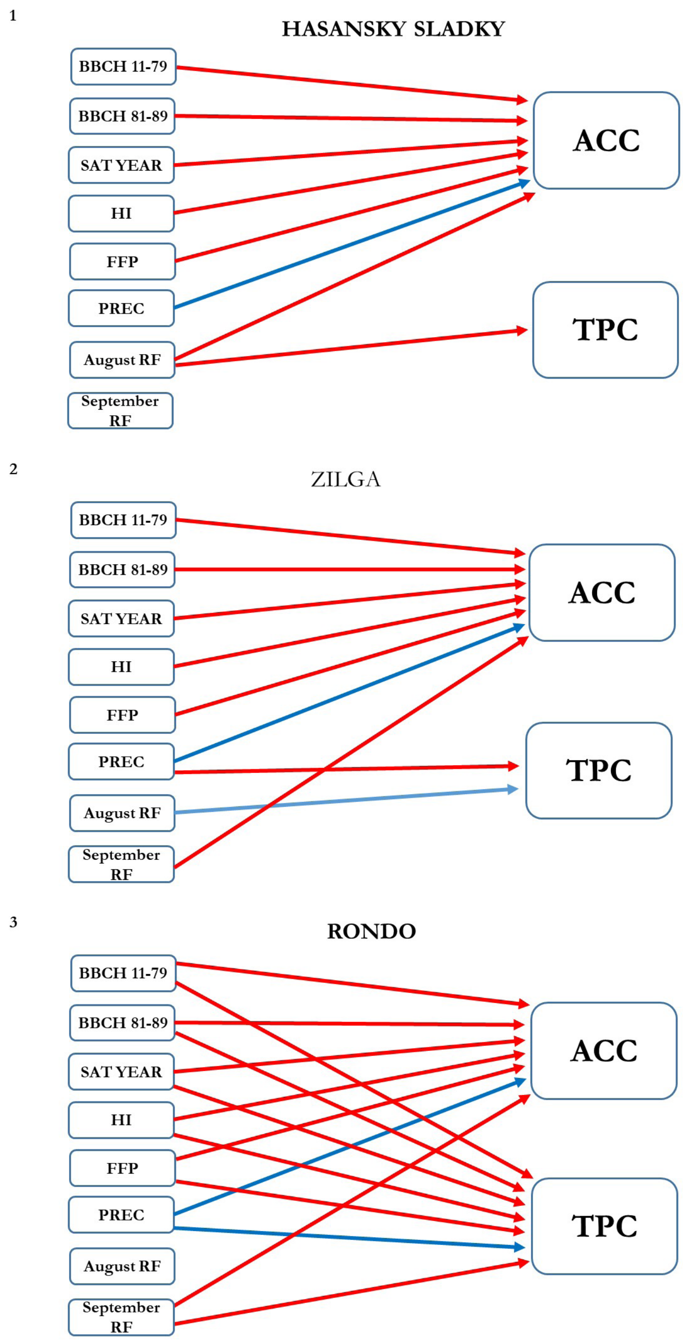


Figure 8. Correlations between phenolic compounds and weather data for grape cultivars 'Hasansky Saldky' (1), 'Zilga' (2), and 'Rondo' (3) in field conditions (2016-2018). Red colour shows positive and blue negative correlation. The significant relationships pointed out in the figure are based on the data in article IV in table 7.

Structure of phenolic maturity parameters in relation to cultivars and weather indicators. PCA showed that the first principal component (PC1) explained 46% of the total variance in the data, and the second principal component (PC2) explained 24% (Fig 2, **IV**). PC1 and PC2 explained 70% of the variance in the data (Fig 2a, b, **IV**). The most important determinants of PC1 were weather-related parameters such as FFP, HI, SAT, SAT BBCH81-89, SAT BBCH 71-79, RH% Sept and Prec (Fig 2a, **IV**). Among fruit quality characteristics, Dp, Mv, and Pt were the most important determinants of PC1. PC2 was primarily determined by ACC, TPC, Cy, Pn, and TAC. It was clearly seen in the PCA map that all polyphenols, especially Dp, Mv, and Pt, were situated in the same area with FFP, HI, SAT (BBCH 71-79, BBCH 81-89, year), and RF (August, September). Among experimental years, the year 2018 was characterised by high values of previously mentioned parameters (Fig 2b, **IV**). The year 2017 distinguished in the opposite site, as it was characterised by high Prec and RH, causing high TAC of fruits. Among cultivars, 'Rondo' was clearly distinguished in the PCA map, situated in the same area with high values of polyphenols. Other cultivars did not have clear distinction in the PCA map.

Effect of viticultural practice on wine grapes. TPC differed significantly between viticultural practices: in the tunnel, TPC of 'Hasansky Sladky' increased in all experimental years, while for 'Zilga' – only in one year (Table 3, **II**). For 'Supaga', the effect of the tunnel was positive in one year, but negative – in the next year. ACC_{spec} in 'Hasansky Sladky' differed between field and tunnel in all experimental years and was significantly higher in the tunnel. In 'Zilga', the effect of viticultural practice emerged in 2013 and 2014. The main effects of viticultural practice and vintage on TPC and ACC_{spec} were significant at $p \leq 0.001$.

In 'Rondo', viticultural practice caused significant variability in ACC_{HPLC} and monoanthocyanidins contents (Table 6, **IV**). ACC_{HPLC} ranged from 447 to 3645 mg 100 g^{-1} in the field and from 1108 to 1618 mg 100 g^{-1} in the tunnel. ACC_{HPLC} and monoanthocyanidins were significantly higher in the grapes cultivated in the field in 2016 and 2018. In 2017, tunnel cultivated grapes had higher monoanthocyanidins content, Dp by 78%, Cy by 26%, Pt by 81%, Pn by 21%, and Mv by 77%. ACC_{HPLC} and monoanthocyanidins were significantly affected by vintage, cultivar, and interaction between them, except for Dp where viticultural practice did not have any significant effect.

6. DISCUSSION

6.1. Technological maturity (I-III)

Cultivar impact on table grapes (I). European Union Regulation provides maturity requirements for *V. vinifera* L. cultivars (OJ L-157 15/06/2011), but these requirements can also be used to assess the quality of hybrids. The lowest allowed SSC is 12 °Brix for 'Alphonse Lavalleé', 'Cardinal', and 'Victoria', 13 °Brix – for all other seeded cultivars, and 14 °Brix – for all seedless cultivars. Seeded cultivars 'Osella', 'Arkadia', 'Swenson Red', 'Supaga', and seedless cultivars 'Mars', 'Somerset Seedless', 'Kosmonavt', 'Canadice' achieved these minimum levels. Jayasena and Cameron (2008) reported that the consumer acceptance increases with SSC increasing from 10 to 20 °Brix. In this experiment, SSC was the lowest in 'Mars', 'Swenson Red', and 'Arkadia' and the highest – in 'Osella', 'Kosmonavt', and 'Somerset Seedless'.

Rapid accumulation of sugars starts in grapes at the beginning of *veraison* and slows as maturity approaches (Bisson 2001, Pedneault *et al*. 2013). The beginning of *veraison* and SSC content depend on the cultivar properties and growth conditions. *Veraison* lasts from 6 to 8 weeks (Plocher and Parke 2008). In this experiment, 'Osella' and 'Kosmonavt' had shorter ripening period. The earliest cultivars were 'Somerset Seedless', 'Kosmonavt', and 'Osella'. In 2014, grapes started to ripen earlier due to higher temperatures in July. Furthermore, higher temperatures enabled SSC to reach the optimum level faster in 2014 than 2013, as demonstrated by SSC stability in the measuring period (less variation than in 2013). Depending on the year, marketing period of table grapes could be from the beginning of August to the end of September.

However, it is important to measure TAC and its ratio to SSC in table grapes. The sweetness of berries depends on the ratio of these parameters. Table grapes SSC/TAC was highly variable and depended on the cultivar. Jayasena and Cameron (2008) found that SSC/TAC was highly correlated with consumer acceptability. Experimental cultivars 'Somerset Seedless' and 'Kosmonavt' had the highest ratio. 'Arkadia' had lower SSC and low TAC, but the ratio was over 26 in both experimental years. It shows that lower sugar content does not always mean sourer berries.

Impact of vintage and cultivar on wine grapes (III). Technological maturity was highly variable and depended on weather conditions. Based on HI classification by Tonietto and Carbonneau (2004) and according to our calculations, experimental areas (Tartu County) located in a very cool region for most experimental years. The exception was 2018, when HI was for the first time in 32 years above 1500 and according to this classification, experimental areas classify as a cool region. This indicates that due temperature conditions, very early and early ripening cultivars could be suitable for cultivation in Estonia. The experiment showed that early ripening 'Hasansky Sladky' is suitable for Estonian very cool weather conditions, but there is a problem with later ripening 'Rondo' and 'Zilga'. Observations show the rise of temperature in Estonia is faster than the global average (Luhamaa *et al.* 2015). According to Luhamaa *et al.* (2014), SAT could rise about 250 °C per vegetation period. It means that, in the future, it is going to be possible to broaden the list of vine cultivars for production.

In a 10-year field experiment, high variability in SSC, TAC, and pH values of hybrid grapevine fruits was found (Table 2, **III**). With hybrid cultivars, significant effect of vintage conditions has been found in other experiments as well (Lisek 2010, Gąstoł 2015). In vinification, recommended values for berry technological maturity are used (Van Schalkwyk and Archer 2000). The recommended °Brix value in grapes for red wines is from 20 to 24, and for white wines – from 19 to 23. The recommended juice pH for white wine is between 3.0 and 3.3, and for red wine – between 3.2 and 3.4 (Van Schalkwyk and Archer 2000). In this research, the obtained pH was at the desired level or near it in every year, but despite that, a problem with sugar and acids content remained. It is in agreement with the literature that *labrusca*-type hybrid grape cultivars like 'Hasansky Sladky' and 'Zilga' might not reach optimal maturity level for wine (Plocher and Parke 2008). These wine grapes can be harvested after SSC has reached °Brix level from 17 to 18, thus before the intensive *labrusca* flavour development. Heymann *et al.* (2013) found that changes in fruit composition are more significant for wine sensory attributes early in ripening than after reaching 24 °Brix.

Based on the experimental results, 'Rondo' did not reach the desired sugar maturity level in field conditions for vinification. 'Hasansky Sladky' reached the desired level and exceeded it in every year, but 'Zilga' – only in three years, and 'Rondo' – in two years. In Southern Finland, 'Zilga' has achieved SSC 19 °Brix (Karvonen 2014b) and 17.9 °Brix (Karvonen 2015). The mean SSC of experimental years in 'Rondo' grapes was 14.9 °Brix, but the mean of the three years in Poland was 17.8 °Brix (Gąstoł 2015). Genotype has influence on grapes sugar concentration (Shiraishi *et al.* 2010). Thus, 'Rondo' has the potential to achieve a higher SSC than was achieved in the field experiment.

SSC accumulation in 'Rondo' was temperature dependent – being enhanced by the mean temperatures and SAT occurring in September, as higher temperature promoted it. 'Hasansky Sladky' and 'Zilga' had one of the highest SSC in 2013 and in 2018. In these years, SAT and HI were high, a FFP – long and Prec level – low. In 2017, all cultivars had one of the lowest SSC. At that time, SAT was lower than 10 years' mean and HI was below 1000. Fruit sugar concentration is higher in higher temperature conditions (Mira de Orduńa 2010). It found confirmation in this study as well.

In most experimental years, TAC was higher than the desirable level for vinification. The recommended TAC value should range from 6.5 to 7.5 g L^{-1} for red wines, and from 7 to 8 g L^{-1} for white wines (Van Schalkwyk and Archer 2000). Cultivars 'Hasansky Sladky' and 'Zilga' reached the desired level in 2018, but 'Rondo' – in none of the experimental years. A decrease in TAC starts at the beginning of ripening and is associated with changes in grape berry respiration (Volschenk *et al.* 2006). Higher temperatures enhance ripening and colourization of berries (due to the degradation of chlorophyll), which promotes the shift from sugar metabolism to MA respiration.

Experimental cultivars reacted to temperature influences differently, because 'Hasansky Sladky' matures earlier compared to 'Zilga' and 'Rondo'. Correlation analyses indicated that warm September for 'Rondo' and longer FFP for 'Rondo' and 'Zilga' had a positive impact, whereas higher HI had no effect on 'Rondo' TAC (Table 4, **III**). This was caused by the peculiarity of the cultivar – 'Rondo' is a later ripening cultivar and needs even higher HI. In a very cool climate, TAC is higher than in warmer climate conditions (Gladstones 1992). Temperatures in

July affected acids accumulation. The recommended temperature for wine grapes is between 20 °C and 25 °C (Dokoozlian 2000). For later ripening 'Rondo', warmer temperatures in autumn were substantial. However, in the experimental years, the weather was significantly cooler and this could cause higher TAC.

Cluster properties such as berry density could have been affected by weather conditions – 'Rondo' and 'Zilga' berries are more tightly in the clusters than those of 'Hasansky Sladky'. Therefore, 'Hasansky Sladky' berries are more open to temperature fluctuation. Biochemical composition of grapes does not depend only on one but on a variety of environmental factors and their interaction (Conde *et al.* 2007, Dai *et al.* 2011). Favourable weather conditions at pre-harvest are low Prec, relatively high temperatures, and long FFP. According to these pre-mentioned conditions, three experimental years out of 10 were compatible for grape cultivating. Therefore, cultivars ripened at different times and weather conditions during grape maturation affected technological maturity accordingly.

Impact of viticultural practice on wine grapes (II, III). 'Hasansky Sladky' SSC achieved the optimum level or exceeded it in both viticultural practices in all three years (**II**). 'Zilga' and 'Supaga' reached the optimum level of °Brix in the tunnel, but not in all years in the field. In the tunnel, TAC was lower, but it was still significantly higher than the recommended level in all experimental cultivars. 'Zilga' achieved the recommended level in both tunnel and field in 2014 and 2015, 'Supaga' – in the tunnel and field in 2014, and 'Hasansky Sladky' – in the field only in 2014.

The cultivation of grapes in the tunnel significantly affected their technological maturity parameters: for all cultivars, in most cases, SSC and juice pH were significantly higher and TAC lower in the tunnel. According to Tarara *et al.* (2008), higher temperatures during ripening decrease TAC and increase juice pH. Higher temperatures could have also affected TAC and juice pH in the current study. In test sites, there were significant differences in air temperatures. In the tunnel, the mean temperature in May was 4.9, 2.8, and 5.9 °C higher (in 2013, 2014 and 2015, respectively) than in the field, and caused earlier growth of vines. In grapes, acids are formed during the first growth period (Kennedy 2002). Our results indicated that higher temperatures in the tunnel (3 to 5 °C in June and 3 to 4 °C in July) reduced acids concentration. The temperature was also higher during *veraison* in August and September. During that period, higher temperature and greater daily temperature fluctuation influenced SSC and caused the reduction of acid levels. Tunnel monthly minimum and maximum temperatures differed almost by 30 °C.

Vintage and viticultural practice had a significant impact on 'Rondo' TA and MA contents (Table 3, **III**). For example, in field conditions, TA content was 35% higher in 2017 than in 2016. The change in the tunnel was 0.2 g L^{-1}. It is found that in a cool climate, the level of MA is usually higher compared to warmer regions (Conde *et al.* 2007). In very cool climate conditions, MA could comprise up to 50% of the total acidity because MA-related respiration is slower in cooler temperature conditions (Volschenk *et al.* 2006). TA was less sensitive to the growing environment, and in 2018, tunnel had not effect on its content. In general, TA concentration in grapes is more constant compared to MA (Volschenk *et al.* 2006). According to literature, high acid content is a consequence of high MA levels (Conde *et al.* 2007), which is a problem in case of cool weather conditions in autumn. It is found that low night temperatures (10–11 °C) around *veraison* period slow down acid degradation, but low night temperatures after *veraison* have an irrelevant effect (Volschenk *et al.* 2006, Gaiotti *et al.* 2018). It is important to monitor MA content, as dry wines made from grapes with too high MA content will have taste sour.

In cool climate conditions, higher MA is considered to be an issue which was also confirmed in this experiment. However, as demonstrated, cultivating vines in the tunnel can reduce MA by 44–65% compared to field conditions. During respiration, MA metabolism rate depends on the temperature: higher temperatures at *veraison* and ripening stages reduce its content (Sweetman *et al.* 2014, Kizildeniz *et al.* 2018). In warmer weather conditions, grape cells increasingly use stored MA to satisfy their energy requirements for berry expansion.

In field conditions, the ratio of TA to MA in grape juice was from 0.7 to 0.8, and in the tunnel – from 1.2 to 2.0 (Table 3, **III**). Kliewer *et al.* (1967) defined four categories to evaluate TA to MA ratio: high malate (below 1.20); moderately high malate (1.21–1.75); average malate (1.76–2.50); low malate (above 2.51). According to this, field cultivated grapes belong to high malate category and tunnel grapes – moderately high malate to average malate category. Grapes harvested under different weather conditions and viticultural practices have diverse profile of organic acids and TA to MA ratio. In field conditions, MA concentration was higher than TA, but in the tunnel, the concentration levels were opposite. It affected TA to MA ratio. The ratio was more or almost twice as high in tunnel cultivated grapes as in field conditions. In wine cultivars, high TA to MA ratio improves wine stability (Liu *et al.* 2007). The ratio is higher in warmer climates, for example in Turkey, where the mean ratio was found to be 2.55 in *V. vinifera* cultivars (Soyer *et al.* 2003), in the Czech Republic, where the ratio differed between cultivars from 1.72 to 3.62 (Pavloušek and Kumšta 2011).

6.2. Phenolic maturity (II, IV)

Impact of vintage and cultivar on wine grapes. In a 9-year field experiment, high variability in TPC and ACC_{spec} of hybrid grapevine fruits was found (Table 4, **IV**). Cultivar, vintage, and their interaction significantly affected the contents. In addition, correlation and PCA analyses indicated that climatic conditions had an impact on phenolic compounds in grapes. Warmer and longer vegetation period increased polyphenols content, as seen in 2018, and more Prec and higher RH decreased it. The effect was cultivar dependent and 'Rondo' differed significantly from the others. TPC in 'Hasansky Sladky' and 'Zilga' had no correlation with temperature-related parameters, but TPC in 'Rondo' had a correlation with weather parameters. All the experimental cultivars had a correlation between ACC_{HPLC} and weather parameters. It may be related to the cultivars' sensitivity to temperature changes. Experimental cultivars had different cluster properties and this could cause variation between cultivars. Berries of 'Rondo' and 'Zilga' were larger and more tightly arranged in the clusters than those of 'Hasansky Sladky' which berries were in thinner clusters with grapes better exposed to light than in tighter clusters. Growth habit of experimental cultivars differed − 'Hasansky Sladky' is more vigorous than 'Zilga'. Vigorous growth and a longer plant growth period affect maturation as well as TPC and ACC_{spec} values. The latter matures the earliest, followed by the ripening of 'Zilga', and finally 'Rondo'. Also, in warmer climates, accumulation of phenolic compounds in *V. vinifera* grapes was influenced by environmental factors (Soubeyrand *et al.* 2014), cultivar (Katalinić *et al.* 2010, Samoticha *et al.* 2017), and *terroir* (Tarko *et al.* 2010).

The differences between vintage could derive from the age of the vines. The vines were three years old at the beginning of the trial and had their first year of harvest, but by the end of the experiment, the vines were 11 years old. As the vines grew older, the trunk thickness and the shoots growth intensity changed and that probably affected the results.

The content of monoanthocyanidins (Dp, Cy, Pt, Pn, and Mv) in all tested hybrid grapes depended on the cultivar and vintage (Table 5, Fig 2, **IV**). Correlation analyses additionally indicated that weather conditions had a correlation with monoanthocyanidins and the effect depended on cultivar. Similar results revealed in an experiment made in a warmer climate with *V. vinifera* cultivars (Ortega-Regules *et al.* 2006), which means that anthocyanin composition depends on the genetic background of *Vitis* species (Liang *et al.* 2008). Still, the differences in anthocyanin composition relate to the cultivar responses to temperature as well (Fernandes De Oliveira *et al.* 2017). In all experimental cultivars, Dp, Pt, and Mv had a positive relationship with temperature-related parameters and a negative one with Prec. Relationship of Cy and Pn depended on cultivar properties.

The order of occurrence of monoanthocyanidins in field-cultivated grapes varied among years. For example, in 'Rondo', it was: Dp > Pn > Mv > Cy > Pt in 2016, Pn > Dp > Mv > Cy > Pt in 2017, and Dp > Mv > Pt > Pn > Cy in 2018. Anthocyanidins give different colours: Cy – crimson, Pn – magenta, Dp – mauve, Pt and Mv – purple. Dp was the dominant monoanthocyanidin in warmer years and Pn – in cooler years. PCA analyses showed that grapes' technological maturity also have influenced their phenolic compounds. Technological maturity parameters of experimental cultivars at harvest varied significantly between years and sometimes did not reach the recommended technological maturity for vinification. Therefore, it can be concluded that the shade of grape colour and intensity of the wine colour from the hybrid grapes in very cool weather conditions vary from year to year and depend on the order of the occurrence of monoanthocyanidins in fruit.

Impact of viticultural practice on wine grapes. There was a significant effect of viticultural practices on phenolic maturity of cultivars 'Hasansky Sladky', 'Zilga', and 'Supaga' (**II**). Differences in experimental results were caused by vineyard conditions, weather factors, and cultivar properties. The soil of the two experimental areas (site 1 and site 3) differed – soil in the field was more fertile than in the tunnel. Site impact on test results was related to the soil and is consistent with previous experiments. Lower phenolic content of grapes is caused by more fertile vineyard soil (de Andrés-de Prado *et al*. 2007), and a decrease in polyphenol content – with a higher nitrogen supply (Heimler *et al*. 2017).

Viticultural practice had a significant effect on ACC_{HPLC} in 'Rondo' (Table 6, **IV**). In 2016 and 2018, ACC_{HPLC} and monoanthocyanidins in 'Rondo' were significantly higher in field cultivated grapes, but in 2017 – in the tunnel. In other experiments, different viticultural practices have been shown to affect anthocyanin profile (Downey *et al*. 2004; Basile *et al*. 2018), as was also confirmed in our experiments. The contents were influenced by weather conditions of experimental years. In 2017, vegetation period was exceptionally cold and rainy (SAT 1981 °C, HI 888 and precipitation 497 mm). In cooler and rainier years, cultivating grapes in the tunnel promoted their maturation. In 2016 and 2018, vegetation period was longer and warmer compared to the average. The year 2018 was exceptionally warm (SAT 2660 °C, HI 1532 and frost-free period 180 days). Elevated temperatures during ripening may reduce anthocyanins accumulation and could partly degrade the previously synthesized compounds (Mori *et al*. 2007, Poudel *et al*. 2009). At *veraison*, the temperatures in the tunnel were higher, and day and night temperature fluctuations were greater (min 5 to 6 °C and max 35 to 40 °C). During this period, higher temperature and greater night and day temperature fluctuation influenced TPC and anthocyanin accumulation. In the tunnel, monthly maximum temperatures were always higher than in the field. Day and night temperature fluctuation was also greater.

The differences in growing conditions between the tunnel and the field could have caused variation in compound bud vitality: whether shoots developed from a larger central primary bud, from smaller secondary buds, or from both at the same time. In the field, the growing season is shorter, and therefore, the vine primary bud may remain less cold hardy. When the primary bud is damaged, the smaller secondary or tertiary bud will break, which will greatly affect yield formation. The second problem is spring frosts. A shoot that has begun to grow from primary bud may be damaged. Then a new shoot begins to grow from the secondary bud or tertiary bud will break, which reduces yield.

7. CONCLUSIONS AND RECOMMENDATIONS

In the present thesis, the effect of vintage, cultivar, and viticultural practices on hybrid grapevines primary and secondary metabolites were investigated and discussed. Based on the results of the experimental work, the following conclusions were drawn.

Impact of cultivar and vintage on table and wine grapes.

- Cultivar properties significantly affected taste parameters of table grapes. In the tunnel, all the seeded and seedless cultivars achieved the minimum requirements of SSC according to the European Union standard for table grapes **(I)**. SSC was highest in 'Osella', 'Kosmonavt', and 'Somerset Seedless'. TAC was highest in 'Canadice', 'Supaga', and 'Swenson Red'. The effect of cultivar on SSC/TAC ratio was significant. Sweeter cultivars were 'Somerset Seedless', 'Kosmonavt', and 'Arkadia'.

- Technological maturity for vinification depended on vintage (**III**). 'Hasansky Sladky' reached the desired SSC level every year in field experiment, but 'Zilga' – only in three years out of ten. SSC in 'Rondo' did not achieve the required level in any experimental year. TAC was a problem in field conditions. It was higher than the recommended level in grapes of 'Hasansky Sladky' in 8 years, in 'Rondo' and 'Zilga' – in 7 years out of 10. In vinification, it is recommended to use techniques to reduce acids, using the measures of maturing in a barrel or applying a suitable yeast.

- Based on 10-year-long data, wine grapes quality depended significantly on the vintage. Based on 32-year-long climate data assessment on macroclimate scale by Huglin's HI, experimental areas in Tartu County are characterised by very cool viticultural zone, which indicates the existence of heliothermal potential for grapes maturation for very early and early ripening cultivars (**III**).

- Significant variability of TPC and ACC in grapes of all experimental cultivars was found (**IV**). Compared to other cultivars, 'Rondo' had higher total polyphenols and anthocyanins contents in most experimental years. In addition, anthocyanidins content was affected by cultivar properties and weather parameters. In every year, most abundant monoanthocyanidins were Mv in 'Hasansky Sladky' and Dp in 'Zilga'.

Impact of viticultural practice on wine grapes.

- In the tunnel, 'Hasansky Sladky', 'Zilga', and 'Supaga' grapes achieved the recommended level of technological maturity parameters – SSC increased and TAC decreased compared to the field **(II, III)**. TAC, TA, and MA were at the appropriate level in tunnel cultivated grapes of 'Rondo' **(III)**. It turned out that TA was at the recommended level, but the problem with high MA revealed in field conditions. In the tunnel, MA content can be reduced.

- Viticultural practices had a significant effect on phenolic maturity of cultivars 'Hasansky Sladky', 'Zilga', 'Supaga' (**II**), and 'Rondo', (**IV**). Growing 'Rondo' in a high polyethylene tunnel increased ACC_{HPLC} during cooler and wetter year, but decreased it in a warmer year. Monoanthocyanidins showed the same tendency.

Depending on the year, table grape harvesting period is from the beginning of August to the end of September. The recommended cultivars are 'Osella', 'Somerset Seedless', 'Kosmonavt', and 'Arkadia'. The ripening of wine grapes varies greatly from year to year, which in turn affects the choice of vinification technology. Our results support the hypothesis that in cold northern climate conditions, different viticultural practices affect the maturity of hybrid grapevines. Early cultivars can be cultivated in the field (eg 'Hasansky Sladky'), but later ripening ones are recommended to be cultivated in the tunnel (e.g. 'Rondo'). The recommendations have also been included in a „List of recommended cultivars in Estonia" (Soovitussortiment 2020). When technological maturity parameters are not at the desirable level for red wine in cool years, e.g. lower SSC, then it is advisable to make wines with lower alcohol content: white, rose or sparkling. Also mixing cultivars can be recommended. For example, 'Hasansky Sladky' had high SSC but low ACC, but in 'Rondo' these parameters were reversed. By mixing the yield of these two cultivars, a better quality raw material for the red wine can be obtained.

Further research objectives:

The results confirmed the need for further research on special cultivars and viticultural practices in Estonia to explore the quality potential of grapes for vinification. In order to obtain a more stable yield of cultivars 'Zilga' and 'Rondo', it is recommended to use viticultural practices that would ensure a yield with more stable quality. Therefore, experiments with different spring covers were started. The goal is to protect the plants from spring frost damage, promote flower fertilization and prolong the growing season. Autumn coverings are also needed to make the harvest time less weather dependent.

From the polyethylene tunnel, it is possible to obtain a yield with stable quality, and it allows to diversify the choice of cultivars. Further research with less winter hardy cultivars will also be continued. Investigation of production residues from the vineyard is important. The leaves of interspecific hybrid grapevine cultivars have a high potential to be used for value addition in developing novel food or livestock feed based products.

REFERENCES

Ananga, A., Georgiev, V., Ochieng, J., Phills, B., Tsolova, V. 2013. Production of Anthocyanins in Grape Cell Cultures: A Potential Source of Raw Material for Pharmaceutical, Food, and Cosmetic Industries. In: Poljuha, D., Sladonja, B. (eds.). The Mediterranean Genetic Code - Grapevine and Olive. Croatia: IntechOpen. pp 324. doi: 10.5772/54592.

Anesi, A., Stocchero, M., Dal Santo, S., Commisso, M., Zenoni, S., Ceoldo, S., Tornielli, G. B., Siebert, T. E., Herderich, M., Pezzotti, M. & Guzzo, F. 2015. Towards a scientific interpretation of the terroir concept: plasticity of the grape berry metabolome. *BMC Plant Biology* 15: 191. doi:10.1186/s12870-015-0584-4

Baiano, A., De Gianni, A., Previtali, M.A., Del Nobile, M.A., Novello, V., De Palma, L. 2015. Effect of defoliation on quality attributes of Nero di Troia (*Vitis vinifera* L.) grape and wine. *Food Research International* 75: 260–269. doi:10.1016/j.foodres.2015.06.007.

Basile, T., Alba, V., Gentilesco, G., Savino, M., Tarricone, L. 2018. Anthocyanins pattern variation in relation to thinning and girdling in commercial Sugrathirteen® table grape. *Scientia Horticulturae* 227: 202–206. doi:10.1016/j.scienta.2017.09.045.

Bisson, L. 2001. In search of optimal grape maturity. *Practical Winery Vineyard*, July/Aug 32–43.

Cheng, G., He, Y.N., Yue, T.X., Wang, J., Zhang, Z.W. 2014. Effects of Climatic Conditions and Soil Properties on Cabernet Sauvignon Berry Growth and Anthocyanin Profiles. *Molecules* 19: 13683–13703. doi:10.3390/molecules190913683.

Chervin, C., Aked, J., Crisosto, Crisosto. 2012. Grapes. In: Rees, D., Farrell, G., Orchard, J. (eds.). Crop Post-Harvest: Science and Technology, First Edition. Blackwell Publishing Ltd., pp 464.

Conde, C, Silva, P, Fontes, N, Dias, A.C.P, Tavares, R.M, Sousa, M.J., Agasse, A., Delrot, S., Gerós, H. 2007. Biochemical changes throughout grape berry development and fruit and wine quality. *Food* 1: 1–22.

Dai, Z.W., Ollat, N., Gomès, E., Decroocq, S., Tandonnet, J.-P., Bordenave, L., Pieri, P., Hilbert, G., Kappel, C., van Leeuwen C., Vivin, P., Delrot, S. 2011. Ecophysiological, genetic, and molecular causes of variation in grape berry weight and composition: a review. *American Journal of Enology and Viticulture* 62: 413–425. doi:10.5344/ajev.2011.10116.

De Andrés-de Prado, R., Yuste-Rojas, M., Sort, X., Andrés-Lacueva, C., Torres, M., Lamuela-Raventós, R.M. 2007. Effect of soil type on wines produced from Vitis vinifera L. cv. Grenache in commercial vineyards. *Journal of Agricultural and Food Chemistry* 55: 779–786. doi:10.1021/jf062446q.

Deloire, A., Vaudour, M.E., Carey, V., Bonnardot, V. van Leeuwen, V. 2005. Grapevine responses to terroir: a global approach. *Journal international des sciences de la vigne et du vin* 39: 149–162. doi:10.20870/oeno-one.2005.39.4.888

Dokoozlian, N.K. 2000. Grape berry growth and development. In: Raisin production manual. Oakland: University of California. Agricultural and Natural Resources Publication. pp. 30–37.

Downey, M.O., Harvey, J.S., Robinson, S.P. 2004. The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Australian Journal of Grape and Wine Research* 10: 55–73. doi:10.1111/j.1755-0238.2004.tb00008.x.

Edelmann, A., Diewok, J., Baena, J.R., Lendl, B. 2003. High-performance liquid chromatography with diamond ATR-FTIR detection for the determination of carbohydrates, alcohols and organic acids in red wine. *Analytical and Bioanalytical Chemistry* 376: 92–97. doi:10.1007/s00216-003-1879-0.

Edo-Roca, M., Nadal, M., Lampreave, M. 2013. How terroir affects bunch uniformity, ripening and berry composition in Vitis vinifera cvs. Carignan and Grenache. *Journal international des sciences de la vigne et du vin* 47: 1–20. doi:10.20870/oeno-one.2013.47.1.1533.

Egnér, H., Riehm, H. and Domingo, W.R. 1960. Untersuchungen uber die chemische Bodenanalyse als Grundlage fur die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung. *Kungliga Lantbrukshögskolans Annaler* 26: 199–215.

Estonian Wine Rout Tour. https://www.visitestonia.com/en/estonian-wine-route-tour. Accessed 11 August 2020.

ETN 003. 2004. Afghan Grapes: Grading for Quality. http://afghanag.ucdavis.edu/a_horticulture/fruitstrees/grapes/factsheetsgrapes/ExtensionToolkitNotes3_GradingforQuality.pdf.pdf/view. Accessed 21 January 2015.

Fernandes De Oliveira, A., Mercenaro, L., Nieddu, G. 2017. Assessing thermal efficiency for berry anthocyanin accumulation in four different sites and field-growing conditions. *Acta Horticulturae* 1188: 181–188. doi:10.17660/ActaHortic.2017.1188.24.

Gaiotti, F., Pastore, C., Filippetti, I., Lovat, L., Belfiore, N., Tomasi, D. 2018. Low night temperature at veraison enhances the accumulation of anthocyanins in Corvina grapes (*Vitis vinifera* L.). *Scientific Reports* 8:8719. doi:10.1038/s41598-018-26921-4.

Gąstoł M. 2015. Vineyard performance and fruit quality of some interspecific grapevine cultivars in cool climate conditions. *Folia Horticulturae* 27: 21–31. doi:10.1515/fhort-2015-0011.

Gatti, M., Frioni, T., Garavani, A., Biagioni, A., Poni, S. 2019. Impact of delayed winter pruning on phenology and ripening kinetics of Pinot Noir grapevines. *BIO Web of Conferences* 13: 04002. doi:10.1051/bioconf/20191304002.

Gladstones J. 1992. Viticulture and environment. Adelaide, Winetitles. pp 310.

Gustafsson, J-G., Mårtensson, A. 2005. Potential for extending Scandinavian wine cultivation. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science* 55: 82–97. doi:10.1080/09064710510029097.

He, F., Liang, N-N., Mu, L., Pan, Q-H., Wang, J., Reeves, M. J., Duan, Chang-Qing. 2012. Anthocyanins and Their Variation in Red Wines I. Monomeric Anthocyanins and Their Color Expression. *Molecules* 17: 1571–1601.

Heimler, D., Romani, A., Ieri, F. 2017. Plant polyphenol content, soil fertilization and agricultural management: a review. *European Food Research and Technology* 243: 1107–1115. doi:10.1007/s00217-016-2826-6.

Heymann H, LiCalzi M, Conversano MR, Bauer A, Skogerson K, Matthews M. 2013. Effects of extended grape ripening with or without must and wine alcohol manipulations on Cabernet Sauvignon wine sensory characteristics. *South African Journal of Enology and Viticulture* 34: 86–99. doi:10.21548/34-1-1084.

Huglin, P. 1978. A new method of evaluating the heliothermal possibilities in the environment of grape culture. *Comptes Rendus l'Académie d'Agriculture de France* 64: 1117–1126.

Hunter, J.J., Deloire, A. 2006. Terroir and vine water relation effects on grape ripening and wine quality of Syrah/R99. Proceedings of the 4th International terroir congress, 3-7 July in Bordeaux and Montpellier, France. p. 110−116.

Hunter, J.J., Volschenk, C.G., Marais, J., Fouché, G.W. 2004. Composition of Sauvignon Blanc grapes as affected by prevéraison canopy manipulation and ripeness level. *South African Journal of Enology and Viticulture* 25: 13–18. doi:10.21548/25-1-2132.

Jayasena, V., Cameron, I. 2008. °Brix/acid ratio as a predictor of consumer acceptability of Crimson Seedless table grapes. *Journal of Food Quality* 31: 736–750.

Jones, G.V., Duff, A.A., Hall, A., Myers, J. 2010. Spatial analysis of climate in winegrape growing regions in the western United States. *American Journal of Enology and Viticulture* 61: 313–326.

Jones, G.V., White, M.A., Cooper, O.R., Storchmann, K. 2005. Climate change and global wine quality. *Climate Change* 73: 319–343. doi:10.1007/s10584-005-4704-2

Kamiloğlu, Ö., Polat, A.A., Durgaç, C. 2011. Comparison of open field and protected cultivation of five early table grape cultivars under Mediterranean conditions. *Turkish Journal of Agriculture and Forestry* 35: 491-499. doi:10.3906/tar-1002-718.

Karp, K., Lajal, T. 2016. Eesti viinamarjaveinide eripära. Aiandusfoorum 2016.

Karvonen, J. 2014a. The annual growth cycle of grapevines in Southern Finland. *Vitis* 53: 175–180. doi:10.5073/vitis.2014.53.175-180

Karvonen, J. 2014b. Vitis cv. Zilga is a vine for the northern temperate climate – Short communication. *Horticultural Science* (Prague) 41: 147–151. doi:10.17221/17/2014-HORTSCI.

Karvonen, J. 2015. Phenolic compounds of grape varieties grown in the northern temperate climate. *International Journal of Agriculture Innovations and Research* 4: 311–316.

Karvonen, J. 2017. Impact of climate change on winegrowing conditions in southernmost Finland (Tuusula). *International Journal of Enology and Viticulture* 4: 169–178.

Katalinić, V., Možina, S.S., Skroza, D., Generalić, I., Abramovič, H., Miloš, M., Ljubenkov, I., Piskernik, S., Pezo, I., Terpinc, P., Boban, M. 2010. Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 Vitis vinifera varieties grown in Dalmatia (Croatia). *Food Chemistry* 119: 715–723. doi:10.1016/j.foodchem.2009.07.019.

Kennedy, J. 2002. Understanding grape berry development. *Practical Winery & Vineyard* 1–5.

Keskin, N., İşçi, B., Gökbayrak, Z. 2013. Effect of cane-girdling and cluster and berry thinning on berry organic acids of four Vitis vinifera L table grape cultivar. *Acta Scientiarum Polonorum Hortorum Cultus* 12: 115–125.

Kivistik, J. 2006. Viinamarjad Eestis. Grapes in Estonia. Ilo, lk 151.

Kivistik, J. 2012. Maalehe viinamarjaraamat. Maalehe Raamat, lk 128.

Kivistik, J. 2017. Maalehe viinamarjaraamat. Hea Lugu, lk 126.

Kizildeniz, T., Pascual, I., Irigoyen, J.J., Morales, F. 2018. Using fruitbearing cuttings of grapevine and temperature gradient greenhouses to evaluate effects of climate change (elevated CO2 and temperature, and water deficit) on the cv. red and white Tempranillo. Yield and must quality in three consecutive growing seasons (2013–2015). *Agricultural Water Management* 202: 299–310. doi:10.1016/j.agwat.2017.12.001.

Kliewer, W.M. 1966. Sugars and organic acids of Vitis vinifera. *Plant Physiology* 41:923–931.

Kliewer, W.M. 1971. Effect of day temperature and light density on concentration of malic and tartaric acids in Vitis vinifera L. grapes. *American Journal of Enology and Viticulture* 96: 372–377.

Kliewer, W.M., Howarth, L., Omori, M. 1967. Concentrations of tartaric acid and malic acids and their salts in Vitis vinifera grapes. *American Journal of Enology and Viticulture* 18: 42–54.

Lambert, M., Meudec, E., Verbaere, A., Mazerolles, G., Wirth, J., Masson, G., Cheynier, V., Sommerer, N. 2015. A high-throughput UHPLC-QqQ-MS method for polyphenol profiling in rosé wines. *Molecules* 20: 7890–7914. doi:10.3390/molecules20057890.

Liang, Z., Cheng, L., Zhong, G.Y., Liu, R.H. 2014. Antioxidant and antiproliferative activities of twenty-four Vitis vinifera grapes. PLoS One, 9: e105146. doi:10.1371/journal.pone.0105146.

Liang, Z., Wu, B., Fan, P., Yang, C., Duan, W., Zheng, X., Liu, C., Li, S. 2008. Anthocyanin composition and content in grape berry skin in Vitis germplasm. *Food Chemistry* 111: 837–844. doi:10.1016/j.foodchem.2008.04.069.

Lisek, J. 2010. Yielding and healthiness of selected grape cultivars for processing in central Poland. *Journal of Fruit and Ornamental Plant Research* 18: 265–272.

Lisek, J. 2012. Winter frost injury of buds on one-year-old grapevine shoots of Vitis vinifera cultivars and interspecific hybrids in Poland. *Folia Horticulturae* 24: 97–103. doi:10.2478/v10245-012-0010-4.

Liu, H.F., Wu, B.H., Fan, P.G., Li, S.H., Li, L.S. 2006. Sugar and acid concentration in 98 grape cultivars analyzed by principal component analysis. *Science of Food and Agriculture* 86: 1526–1536.

Liu, H.F., Wu, B.H., Fan, P.G., Xu, H.Y., Li, S.H. 2007. Inheritance of sugars and acids in berries of grape (Vitis vinifera L.). *Euphytica* 153: 99–107. doi:10.1007/s10681-006-9246-9.

Lorenz, D.H., Eichhorn, K.W., Bleiholder, H., Klose, R., Meier, U., Weber, E. 1994. Phenological growth stages of the grapevine (Vitis vinifera L. ssp. vinifera)—codes and descriptions according to the extended BBCH. *Die Weinwissenschaft – Viticultural and Enological Sciences* 49: 66–70.

Luhamaa, A., Kallis, A., Mändla, K., Männik, A., Pedusaar, T., Rosin, K. 2014. Eesti tuleviku kliima stsenaariumid aastani 2100. Lepingulise töö aruanne projekti "Eesti riikliku kliimamuutuste mõjuga kohanemise strateegia ja rakenduskava ettepaneku väljatöötamine" lisana. Keskkonnaagentuur 94 pp.

Luhamaa, A., Kallis, A., Mändla, K., Männik, A., Pedusaar, T., Rosin, K. 2015. Eesti tuleviku kliimastsenaariumid aastani 2100. Lõpparuanne. Keskkonnaagentuur, lk 35.

Maante, M., Vool, E., Karp, K. 2016. Effect of defoliation on grape maturity parameters. *Sodininkyste ir Darzininkyste* 35: 21–35.

Martin, S.R., Dunn, G.M. 2000. Effect of pruning time and hydrogen cyan amide on budburst and subsequent phenology of Vitis vinifera

L. variety Cabernet Sauvignon in central Victoria. *Australian Journal of Grape and Wine Research* 6: 31–39. doi:10.1111/j.1755-0238.2000.tb00159.x

Miidla, H. 1964. Viinamarjakasvatus. Eesti Riiklik Kirjastus, Tallinn, 136 lk.

Mira de Orduña, R. 2010. Climate change associated effects on grape and wine quality and production. *Food Research International* 43: 1844–1855. doi:10.1016/j.foodres.2010.05.001.

Mori, K., Goto-Yamamoto, N., Kitayama, M., Hashizume, K. 2007. Loss of anthocyanins in red-wine grape under high temperature. *Journal of Experimental Botany* 58: 1935–1945,.doi:10.1093/jxb/erm055.

Muñoz-Robredo, P., Robledo, P., Manriques, D., Molina, R., Defilippi, B.G. 2011. Characterization of sugars and organic acids in commercial varieties of table grapes. *Chilean Journal of Agricultural Research* 71: 452–458.

Nelson, K.E. 1985. Harvesting and handling California table grapes for market. Second Edition, UCANR Publications, pp 67.

Nogales-Bueno, J., Hernándes-Hierro, J.M., Rodrigues-Pulido, F.J. 2014. Determination of technological maturity of grapes and total phenolic compounds of grapes skins in red and white cultivars during ripening by near infrared hyperspectral image: A preliminary approach. *Food Chemistry* 152: 586–591.

OIV. 2008. Resolution VITI 1/2008. OIV Standard on Minimum Maturity Requirements for Table Grapes. Organisation Internationale de la Vigne et du Vin, Paris. ftp://ftp.fao.org/codex/meetings/cac/cac36/if36_05e.pdf. Accessed 21 January 2015.

OJ L-157 15/06/2011. Marketing standards for fresh fruits and vegetables. http://exporthelp.europa.eu/update/requirements/ehir_eu13_02v001/eu/auxi/eu_mktfrveg_annex1b_r543_2011_grapes.pd. Accessed 10 December 2014

Ortega-Regules, A., Romero-Cascales, I., Lopes-Roca, J.M., Ros-Garcia, J.M., Gomez-Plaza, E. 2006. Anthocyanin fingerprint of grapes: environmental and genetic variations. *Journal of the Science of Food and Agriculture* 86: 1460–1467. doi:10.1002/jsfa.2511.

Pavloušek, P., Kumšta, M. 2011. Profiling of primary metabolites in grapes of interspecific grapevine varieties: sugars and organic acids. *Czech Journal of Food Sciences* 29: 361–372. doi:10.17221/257/2010-CJFS.

Pedastsaar, P., Vaher, M., Kulp, M., Kaljurand, M., Karp, K., Raal, A., Karathanos, V., Püssa, T. 2014. Chemical composition of red wines made from hybrid grape and common grape (Vitis vinifera L.) cultivars. *Proceedings of the Estonian Academy of Science*s 63: 444–453. doi:10.3176/proc.2014.4.10.

Pedneault, K., Dorais, M., Angers, P. 2013. Flavor of cold-hardy grapes: impact of berry maturity and environmental conditions. *Agricultural and Food Chemistry* 61, 10418–10438.

Pereira, G.E., Gaudillere, J.P., van Leeuwen, C., Hilbert, G., Maucourt, M., Deborde, C. 2006. H1-NMR metabolite fingerprints of grape berry: Comparison of vintage and soil effects in Bordeaux grapevine growing areas. *Analytica Chimica Acta* 563: 346–352. doi:10.1016/j.aca.2005.11.007

Plocher, T., Parke, B. 2008. Growing grapes and making wine in cool climate. Second Edition, Northern Winework, 208 pp.

Poudel, P.R., Mochioka, R., Beppu, K., Kataoka, I. 2009. Influence of Temperature on Berry Composition of Interspecific Hybrid Wine Grape 'Kadainou R-1' (Vitis ficifolia var. ganebu × V . vinifera 'Muscat of Alexandria'). *Journal of the Japanese Society for Horticultural Science* 78: 169–174. doi:10.2503/jjshs1.78.169.

Rätsep, R., Karp, K., Vool, E., Tõnutare, T. 2014. Effect of pruning time and method on hybrid grapevine (Vitis sp.) "Hasanski Sladki" berry maturity in a cool climate conditions. *Acta Scientiarum Polonorum Hortorum Cultus* 13: 99−112.

Revilla, E., Losada, M., Gutiérrez, E. 2016. Phenolic Composition and Color of Single Cultivar Young Red Wines Made with Mencia and Alicante-Bouschet Grapes in AOC Valdeorras (Galicia, NW Spain). *Beverages* 2: 18. doi:10.3390/beverages2030018.

Ruffner, H.P. 1982. Metabolism of tartaric and malic acids in Vitis: a review – part A. *Vitis* 21: 247–259.

Samoticha, J., Wojdyło, A., Golis, T. 2017. Phenolic composition, physicochemical properties and antioxidant activity of interspecific hybrids of grapes growing in Poland. *Food Chemistry* 215: 263–273. doi:10.1016/j.foodchem.2016.07.147.

Shiraishi, M., Fujishima, H., Chijiwa, H. 2010. Evaluation of table grape genetic resources for sugar, organic acid, and amino acid composition of berries. *Euphytica* 174: 1–13.

Soovitussortiment. 2013. Eesti puuvilja- ja marjakultuuride soovitussortiment – Viinapuu. http://aianduskool.ee/jaan/puuv13/sort2013/sortiment_2013.pdf Accessed 10 August 2020.

Soovitussortiment. 2020. Eesti puuvilja- ja marjakultuuride soovitussortiment – Viinapuu. https://d5q4d9a8.stackpathcdn.com/wp-content/uploads/2020/10/Eesti-puuvilja-ja-marjakultuuride-soovitussortiment.pdf Accessed 14 April 2021.

Soubeyrand, E., Basteau, C., Hilbert, G., van Leeuwen, C., Delrot, S., Gomès, E. 2014. Nitrogen supply affects anthocyanin biosynthetic and regulatory genes in grapevine cv. Cabernet-Sauvignon berries. *Phytochemistry* 103: 38–49. doi:10.1016/j.phytochem.2014.03.024.

Soyer, Y., Koca, N., Karadeniz, F. 2003. Organic acid profile of Turkish white grapes and grape juices. *Journal of Food Composition and Analysis* 16: 629–636. doi:10.1016/S0889-1575(03)00065-6.

Sustainable adaptation of typical EU farming systems to climate change. Baseline report for the 4 main EU Climate Risk Regions. 2017. https://agriadapt.eu/documents/. Accessed 1 March 2018.

Sweetman, C., Deluc, L.G., Cramer, G.R., Ford, C.M., Soole, K.L. 2009. Regulation of malate metabolism in grape berry and other developing fruits. *Phytochemistry* 70: 1329–1344. doi:10.1016/j.phytochem.2009.08.006.

Sweetman, C., Sadras, V.O., Hancock, R.D., Soole, K.L., Ford, C.M. 2014. Metabolic effects of elevated temperature on organic acid degradation in ripening Vitis vinifera fruit. *Journal of Experimental Botany* 65: 5975–5988. doi:10.1093/jxb/eru343.

Tang, K., Liu, T., Han, Y., Xu, Y., Li, J.M. 2017. The Importance of Monomeric Anthocyanins in the Definition of Wine Colour Properties. *South African Journal for Enology and Viticulture* 38: 1. doi: 10.21548/38-1-661.

Tarara, M.J., Lee, J., Spayd, E.S. & Scagel, F.C. 2008. Berry temperature and Solar Radiation alter acylation, propotion, and concentration of anthocyanin in Merlot grapes. *American Journal of Enology and Viticulture* 59, 235-247.

Tarko, T., Duda-Chodak, A., Sroka, P., Satora, P., Jurasz, E. 2010. Polish wines: characteristics of cool-climate wines. *Journal of Food Composition and Analysis* 23: 463–468. doi:10.1016/j.jfca.2010.01.009.

Tarricone, L., Masi, G., Amendolagine, A., Di Gennaro, D., Suriano, S., Savino, M., Bavaresco, L., Gardiman, M. 2013. Terroir influence on viticultural and oenological characteristics of Vitis vinifera cv. Negro Amaro. Proceedings of the 36th World Congress of vine and wine, in 2-7 June in Bucharest, Romania. http://hdl.handle.net/10807/61704.

Tonietto, J., Carbonneau, A. 2004. A multicriteria climatic classification system for grape-growing regions worldwide. *Agricultural and Forest meteorology* 124: 81–97. doi:10.1016/j.agrformet.2003.06.001.

Van Leeuwen, C., Seguin, G. 2006. The concept of terroir in viticulture. *Journal of Wine Research* 17: 1-10. doi:10.1080/09571260600633135.

Van Schalkwyk, H., Archer, E. 2000. Determining optimum ripeness in wine grapes. *Wineland* 130:90–91.

VitiNord 2018. http://www.vitinord2018.org/about/. Accessed 7 August 2020

Volschenk, H., van Vuuren, H.J.J., Viljoen-Bloom, M. 2006. Malic acid in wine: origin, function and metabolism during vinification. *South African Journal for Enology and Viticulture* 27: 123–136. doi:10.21548/27-2- 1613.

Wojdyło, A., Samoticha, J., Nowicka, P., Chmielewska, J. 2018. Characterisation of (poly)phenolic constituents of two interspecific red hybrids of Rondo and Regent (Vitis vinifera) by LC–PDA–ESI-MS QTof. *Food Chemistry* 239: 94–101. doi:10.1016/j.foodchem.2017.06.077.

Wrolstad, R.E., Acree, T.E., Decker, E.A., Penner, M.H., Reid, D.S., Schwartz, S.J., Shoemaker, C.F., Denise, M.S. & Sporns, P. (eds.). 2005. Handbook of Food Analytical Chemistry. Pigments, colorants, flavors, texture, and bioactive food components. New Jersey: John Wiley & Sons, pp 624. doi:10.1002/0471709085.

Yang, J., Martinson, T.E., Liu, R.H. 2009. Phytochemical profiles and antioxidant activities of wine grapes. *Food Chemistry* 116: 332–339. doi:10.1016/j.foodchem.2009.02.021.

Zhu, L.; Zhang, Y.; Lu, J. 2012. Phenolic contents and compositions in skins of red wine grape cultivars among various genetic backgrounds and originations. *International Journal of Molecular Sciences* 13: 3492–3510. doi:10.3390/ijms13033492.

SUMMARY IN ESTONIAN

VIINAPUU (*VITIS* SP) SAAGI KÜPSUSNÄITAJAD

Sissejuhatus

Jahedama kliimaga riikides tuntakse viinamarjade kasvatamise vastu üha rohkem huvi. Neid piirkondi on peetud sobimatuks lühikese vegetatsiooniperioodi ja muutlike ilmastikuolude tõttu (Kennedy 2002, Karvonen 2014a). Traditsioonilistes veinimaades näitab vegetatsiooniperiood soojenemistendentsi (Jones *et al.* 2005). Muutusi on täheldatud ka külmema kliimaga Eestis (Luhamaa *et al.* 2015). Selle tulemusena võib suureneda vegetatsioonperioodil aktiivsete temperatuuride summa 250 °C võrra (Luhamaa *et al.* 2014), mis on viinamarjade kasvatamise jaoks soodne.

Eestis alustati viinapuude uuringutega juba 1950. aastatel (Miidla 1964). Sellel ajal kasvatati peamiselt *V. vinifera* sorte. Tänapäeval kasvatatakse peamiselt hübriidsorte, mis on külma- ja talvekindlamad. Viimastel aastatel on kogunud viinamarjakasvatus tootjate seas järjest enam populaarsust, kuid seni on vähe teaduspõhist teavet viinapuu erinevate kasvatustehnoloogiate, sortide ja saagi kvaliteedinäitajate kohta. Eesti Maaülikoolis alustati sordivõrdluskatsetega 2003. aastal erinevates tootmisistandikes Tartu-, Võru- ning Saaremaal. Perspektiivikateks osutusid punase veini sordid 'Hasanski Sladki', 'Zilga' ja 'Rondo' ning valge veini jaoks 'Supaga'. Nende sortidega rajati 2007. aastal Rõhu katseaeda viinamarjaistandik. Sellest ajast alates on olnud uuringute tähelepanu keskpunktis veiniviinamarjade kvaliteet. Avamaa tingimustes osutus probleemseks sordiks 'Rondo'. Seetõttu tehti ka veinimarjade katsed kiletunnelites.

Viinamarjakasvatuses ja veini valmistamisel sõltub töö tulemus mitmest tegurist. Seetõttu kasutatakse rahvusvahelist terminit *terroir*, mis koondab kõik mõjurid (Deloire *et al.* 2005, Tarricone *et al.* 2013, Edo-Roca *et al.* 2013). See hõlmab sordi, ilmastiku, mullatingimuste ja inimteguri (kasvatustehnoloogia) koosmõju (van Leeuwen ja Seguin 2006, Anesi 2015). *Terroir*-veinid peegeldavad lisaks istanduse asukoha ja kasvatustehnoloogia eripäradele ka veinimeistri käekirja. Seega algab *terroir*-veini tootmine istandiku asukoha valikuga. Pereira *et al.* (2006)

leidsid, et marja esmaste ja teiseste ainevahetussaaduste sisaldust mõjutab rohkem kliima kui mullatingimused. Sellest lähtudes on Eestis valmistatud vein unikaalne ja sellele regioonile spetsiifiline. Seetõttu on vaja katseid, mis aitaksid Eesti viinamarjakasvatajal ja veinimeistril leida sobivaid sorte ning rõhutada Eesti veini eripära *terroir*'i aspektist (kasvatustehnoloogiad, sortide valik, kasvukeskkond ja kliima).

Kvaliteetse saagi saamiseks määratakse viinamarjade tehnoloogilist ja fenoolset küpsust. Lauamarjade puhul on tähtsad tehnoloogilise küpsuse näitajad marjamahla kuivaine ja orgaaniliste hapete sisaldus ning nende suhtarv. Eelnimetatutest on Euroopa Liidu kvaliteedinõuetes fikseeritud mahla kuivainesisaldus (OJ L-157 15/06/2011). Veinimarjade tehnoloogilise küpsuse näitajad on marjamahla kuivaine-, orgaaniliste hapete sisaldus ja pH. Nende järgi otsustatakse korjeaeg ja veini valmistamise viis. Eestis on probleemiks olnud mahla vähene kuivaine ja orgaaniliste hapete suur sisaldus. Happesisalduse reguleerimiseks on vajalik analüüsida ka hapete koostist. Kuivale veinile annab ebameeldiva teravhapu maitse just liigne õunhappesisaldus. Viinhappesisaldus on marjamahlas püsivam (Ruffner 1982, Volschenk *et al.* 2006), samas kui õunhappesisaldus sõltub rohkem saagiaastal valitsevast temperatuurist (Sweetman *et al.* 2014, Kizildeniz *et al.* 2018). Tähtsad fenoolse küpsuse näitajad on polüfenoolide ja antotsüaanide sisaldus ning antotsüaanide profiil. Nende hulgast sõltub eelkõige veini värvuse intensiivsus ja toon, kuid polüfenoolid on ka tähtsad antioksüdandid, mis mõjutavad veini tervislikkust.

Püstitatud hüpoteesid ja eesmärgid

Laua- ja veiniviinamarjade biokeemiline koostis on mõjutatud sordi omadustest, saagiaastast ja kasvatustehnoloogiast. Enamus uuringuid on tehtud erinevate *V. vinifera* sortidega. Vähe on informatsiooni viinapuu hübriidsortide ja nende saagi küpsuse näitajate varieeruvuse kohta jahedas kliimas. Eelnevate uuringute ja katsete põhjal püstitati järgmised hüpoteesid:

- Eestis saab kasvatada lauaviinamarju vastavalt turustusstadardis kehtestatud nõuetele.
- Viinamarja hübriidsortide esmaste ja teiseste ainevahetussaaduste sisaldus sõltub Eesti ilmastikust, sordi omadustest ning

kasvatustehnoloogiatest. Samas ei ole teada, kui oluline see mõju on ja kas soovitatud küpsuse näitajate tase saavutatakse.

- Veiniviinamarjade kasvatamine kiletunnelis mõjutab positiivselt nende tehnoloogilise ja fenoolse küpsuse näitajaid.

Doktoritöö eesmärk oli välja selgitada järgmist:

- Lauaviinamarjade sordi omaduste mõju marjamahla kuivaine **(I)** ja orgaaniliste hapete sisaldusele ning nende suhtarvule.

- Saagiaasta mõju hübriidsortide 'Hasanski Sladki', 'Zilga' ja 'Rondo' saagi tehnoloogilise ning fenoolse küpsuse näitajatele **(III, IV)**.

- Kasvatustehnoloogiate mõju hübriidsortide 'Hasanski Sladki', 'Zilga', 'Rondo' ja 'Supaga' saagi tehnoloogilise ning fenoolse küpsuse näitajatele **(II, III, IV)**.

Katsematerjal ja metoodika

Doktoritöös on kasutatud 2009. kuni 2018. aastal avamaalt ja kiletunnelitest kogutud katseandmeid (tabel 4). Avamaa andmed koguti Eesti Maalikooli Rõhu katseistandikust (58° 21' N, 26° 31' E) ning kiletunneli andmed Nõo (58° 17' N, 26° 33' E) ja Lüüste (58° 37' N, 25° 8' E) istandikest.

Tehtud analüüsid

Arvutati Eesti heliotermiline indeks (HI, Huglin 1978, **III–IV**). Arvutamisel võeti arvesse päeva pikkuse koefitsienti, mis on 58. laiuskraadil 1,09.

Fenoloogilised vaatlused viidi katsekohtades läbi olenevalt aastast aprillist kuni oktoobrini. Saagi analüüsid tehti võrse esimesest vilikonnast. Katseaasta lõpus kaaluti vilikonna mass, loeti marjade arv ning arvutati marja mass saja marja keskmisena (**IV**).

Tabel 4. Ülevaade doktoritöö aluseks olnud katsetest.

	Sort	Mõju-tegur	Näitajad	Aasta	Asukoht
I	'Osella' 'Kosmonavt' 'Mars' 'Swenson' 'Red' 'Somerset' 'Seedless' 'Canadice' 'Arkadia' 'Supaga'	Sort	SSC, TAC, SSC/TAC	2013–2014	Lüüste
II	'Hasanski Sladki', 'Zilga', 'Supaga'	Kasvu-koht	SSC, TAC, SSC/TAC, pH, TP, AC_{spec}	2013–2015	Lüüste, Rõhu
III	'Hasanski Sladki' 'Zilga' 'Rondo'	Aasta, kasvukoht	HI, SSC, TAC_{titr}, TAC_{spec}, pH, TA, MA, TA/MA	2009–2018	Nõo, Rõhu
IV	'Hasanski Sladki' 'Zilga' 'Rondo'	Aasta, kasvukoht	HI, AC_{spec}, AC_{HPLC}, TP, Cy, Dp, Pn, Pt, Mv, CW, NBC, BW	2010–2018	Nõo, Rõhu

SSC – marjamahla kuivaine, TAC – orgaanilised happed, SSC/TAC – marjamahla kuivaine ja orgaaniliste hapete suhtarv, TP – polüfenoolid, AC – antotsüaanid, TA – viinhape, MA – õunhape, TA/MA – viin- ja õunhappe suhtarv, Cy – tsüanidiin-3-O-glükosiid, Dp – delfinidiin-3-O-glükosiid, Pn – peonidiin-3-O-glükosiid, Pt – petunidiin-3-O-glükosiid, Mv – malvidiin-3-O-glükosiid, NBC – marjade arv vilikonnas, BW – marja mass, CW – vilikonna mass, HI – heliotermiline indeks.

Fenoloogilised vaatlused viidi katsekohtades läbi olenevalt aastast aprillist kuni oktoobrini. Saagi analüüsid tehti võrse esimesest vilikonnast. Katseaasta lõpus kaaluti vilikonna mass, loeti marjade arv ning arvutati marja mass saja marja keskmisena (**IV**).

Tehnoloogilise küpsuse näitajad. Marjamahla kuivaine (°Brix) määrati portatiivse refraktomeetriga (Atago Pocket Refractometer Pal-1, Jaapan) (**I**). Mõõdeti 08. augustist 20. septembrini 2013. aastal ja 07. augustist 12. septembrini 2014 aastal. Teistes katsetes määrati marjamahla kuivainesisaldus saagi korjamisel (**II–III**). Orgaaniliste hapete sisaldus määrati tiitrimeetriliselt (Mettler Toledo EasyPlus Titration titraator) (**I–III**). Mahla kuivaine ja orgaaniliste hapete suhe arvutati eelnimetatud näitajate põhjal ning väljendati suhtarvuna (**I**). Viinhappe, õunhappe ja hapete üldsisaldus määrati FT-IR-i spektromeetrilisel meetodil (ALPHA, Bruker Optics, Saksamaa) (**III**). Arvutati suhtarv viinhappe ja õunhappe vahel. Viinamarjamahla pH mõõdeti pH-meetriga (HD 2156.1, Delta OHM) (**II–III**).

Fenoolse küpsuse näitajad. Polüfenoolide üldsisaldus määrati viinamarja kestadest spektrofotomeetriliselt (UVmini-1240 Shimadzu, Jaapan) Folin-Ciocalteau meetodil (**II, IV**). Antotsüaanide üldsisaldus määrati viinamarjakestadest spektrofotomeetriliselt pH-diferentsiaalmeetodil (**II**, **IV**). Antotsüaanide profiil määrati kromatograafiliselt (Nexera X2 Shimadzu, Jaapan) polüfenoolide profileerimise meetodil (**IV**).

Ilmastik. Ilmastiku andmed pärinevad Eesti keskkonnaagentuuri riigi ilmateenistusest Tartu-Tõravere ja Kaansoo meteoroloogiajaamadest, Tartu Ülikooli keskkonnafüüsika labori ilmajaamast ning Agri4cast-i andebaasist.

Tulemuste kokkuvõte

Saagiaasta ja sordi mõju viinamarjasaagile.

- Sordi omadused mõjutasid märgatavalt lauaviinamarjade maitse näitajaid (**I**). Kõik seemnetega ja seemneteta sordid saavutasid minimaalse Euroopa Liidu standardis kehtestatud mahla kuivainesisalduse. Mahla kuivainesisaldus oli suurim sortides 'Kosmonavt', 'Somerset Seedless' ja 'Osella'. Orgaaniliste hapete sisaldus oli suurim sortides 'Canadice', 'Supaga' ja 'Swenson Red'. Sordil oli oluline mõju mahla kuivaine ja orgaaniliste hapete suhtarvule. Magusaimad marjad olid sortidel 'Somerset Seedless', 'Kosmonavt' ja 'Arkadia'.

- Veiniviinamarjade tehnoloogiline küpsus sõltus saagiaastast (**III**). Veini valmistamiseks vajaliku marjamahla kuivainesisalduse saavutas avamaal 'Hasanski Sladki' kümnel, 'Zilga' kolmel, kuid 'Rondo' mitte ühelgi saagiaastal. Suur orgaaniliste hapete sisaldus osutus avamaal probleemiks. Sordil 'Hasanski Sladki' jäi see soovitatavast vahemikust suuremaks kaheksal aastal, sortidel 'Rondo' ja 'Zilga' seitsmel aastal kümnest. Veini valmistamisel on soovitatav kasutada võtteid, mis vähendaksid hapete sisaldust. Selleks võib kasutada veini laagerdamist vaadis või sobilikku pärmi.

- Kümme aastat kestnud katse põhjal selgus, et viinamarjade saagi kvaliteet sõltus suurel määral saagiaasta ilmastikutingimustest. Põhinedes heliotermilise indeksi mikrokliima skaala 32 aasta kliimaandmete analüüsil, kuulub Tartumaa väga jahedasse viinamarjakasvatuse piirkonda (**III**). 2018. aastal oli heliotermiline indeks esimest korda üle 1500 ja selle järgi kuulus see ala jahedasse piirkonda. See näitab, et Eestis saab kasvatada avamaa tingimustes väga varajasi või varajasi sorte, nagu 'Hasanski Sladki' ja 'Zilga'.

- Polüfenoolide ja antotsüaanide üldsisaldus varieerus avamaal oluliselt üheksal katseaastal kõigis sortides (**IV**). Enamikul katseaastatel oli sordil 'Rondo' suurim polüfenoolide ja antotsüaanide üldsisaldus. Varieeruvus sõltus sordist ja seda mõjutasid saagiaasta ilmastikutingimused. Sordi omadused ja saagiaasta tingimused mõjutasid ka antotsüanidiinide sisaldust. Sort 'Hasanski Sladki' sisaldas igal aastal kõige enam malvidiin-3-O-glükosiidi ning 'Zilga' delfinidiin-3-O-glükosiidi. Antotsüaanide kogusisaldus oli igal aastal suurim sordil 'Rondo'.

Kasvatustehnoloogia mõju veiniviinamarjadele.

- Võrreldes avamaaga saavutasid sordid 'Hasanski Sladki', 'Zilga' ja 'Supaga' kiletunnelis soovitatava tehnoloogilise küpsuse – mahla kuivainesisaldus suurenes ja orgaaniliste hapete sisaldus vähenes (**II**, **III**). Samuti oli sobiv orgaaniliste hapete, õun- ja viinhappesisaldus sordil 'Rondo' (**III**). Selgus, et avamaal oli viinhappesisaldus soovitataval tasemel, aga probleemiks osutus õunhappesisaldus. Tunnelis kasvatamisel saab viinamarjades õunhappesisaldust vähendada.

- Sortide 'Hasanski Sladki', 'Zilga', 'Supaga' (**II**) ja 'Rondo' (**IV**) fenoolset küpsust mõjutas oluliselt kasvatustehnoloogia. Sordil 'Rondo' vähenes kiletunnelis antotsüaanide sisaldus soojal aastal, kuid suurenes jahedal ja vihmasel aastal. Sama tendentsi näitasid antotsüanidiinid.

Sõltuvalt aastast kestab lauaviinamarjade saagiperiood augusti algusest kuni septembri lõpuni. Soovitatavad sordid on 'Osella', 'Somerset Seedless', 'Kosmonavt' ja 'Arkadia'. Veiniviinamarjade puhul näitasid katsetulemused, et viinamarjade valmimine on aastati väga erinev, mis omakorda mõjutab veinitehnoloogia valikut. Meie tulemused toetavad hüpoteesi, et põhjamaises jahedas kliimas mõjutavad erinevad kasvatustehnoloogiad viinamarjade valmimist, mida tuleb arvestada veinitehnoloogia valikul. Avamaa tingimustes on soovitatav kasvatada varajasi sorte (näiteks 'Hasanski Sladki'), kuid kiletunnelis hilisemaid sorte (näiteks 'Rondo'). Soovitused on sisse viidud ka Eesti soovitussortimenti (Soovitussortiment 2020). Kui tehnoloogilise küpsuse näitajad ei ole punase veini valmistamiseks soovitataval tasemel, siis võib teha jahedamatel aastatel väiksema alkoholisisaldusega roosat, valget või vahuveini. Samuti võib soovitada sortide segamist. Näiteks sordil 'Hasanski Sladki' oli suur marjamahla kuivainesisaldus, aga väike antotsüaanide sisaldus. 'Rondol' seevastu olid need näitajad vastupidised. Nende kahe sordi segamisel saame veini valmistamiseks parema kvaliteediga tooraine.

Edasist uurimist vajavad teemad

Stabiilsema saagi saamiseks on Tartumaal vajalik 'Zilga' ja 'Rondo' puhul kasutada tehnoloogiaid, mis tagaksid stabiilsema kvaliteediga saagi. Seetõttu alustati katseid erinevate katmisviisidega, et kaitsta taimi kevadiste külmakahjustuste eest. Samuti alustati sügiskatete uurimisega, et saagi korjamine sõltuks vähem ilmastikutingimustest. Stabiilsema kvaliteediga saagi saab kiletunnelist ning edasi jätkatakse uuringuid külmaõrnemate ja hilisemate sortidega. Tähtis on uurida edasi ka viinamarjakasvatuses tekkinud tootmisjääke. Liikidevaheliste hübriidsortide viinapuude lehtedel on potentsiaal uute toidu- või loomasöödalisandite väljatöötamisel.

ACKNOWLEDGEMENTS

I would like to express my greatest gratitude to my supervisor Professor Kadri Karp. Thank you for your constructive guidance, support and understanding over the years. I would like to thank my supervisors Ele Vool for choosing me to fulfill this assignment and Associate professor Leila Mainla for the constructive guidance. Thank you very much!

I am greatly thankful to my colleagues from the Chair of Horticulture for their support and that they form supportive environment for working on my thesis. I would like to thank colleagues from Polli Horticultural Research Center and especially Reelika Rätsep for supervising in grapes analyses and critical review of my articles. As well as for help in the laboratory by Alar Aluvee and Hedi Kaldmäe. Järiste winery and Saare-Tõrvaaugu vineyard for the cooperation. Thank you!

Olen tänulik oma perekonnale, eriti vanematele ja abikaasale, kes on toetanud minu õpinguid läbi kõigi nende aastate. Suur tänu teile!

The Estonian Environment Agency, Agri4cast and Laboratory of Environmental Physics of Tartu University are gratefully acknowledged for weather data.

The present research was supported by Estonian Science Foundation [grant number 9363], Target Funding project SF0170057s09 and Institutional Funding project IUT36-2, "Innovation Cluster" of Estonian Rural Development Plan (ERDP Action 16 'Cooperation') 2014–2020, financially supported by European Agricultural Fund for Rural Development (EAFRD).

These studies were also supported by Internationalization Programme DoRa. Programme DoRa is carried out by Archimedes Foundation.

I

ORIGINAL PUBLICATIONS

Maante, M., Vool, E., Rätsep, R., Karp, K. 2015. The effect of genotype on table grapes soluble solids content. *Agronomy Research* 13(1): 141−147.

Agronomy Research **13**(1), 141–147, 2015

The effect of genotype on table grapes soluble solids content

M. Maante*, E. Vool, R. Rätsep and K. Karp

Department of Horticulture, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 64, EE51014 Tartu, Estonia;
*Correspondence: mariana.maante@student.emu.ee

Abstract. Sugar concentration in fresh consumed table grapes is mainly connected with technological maturity and primarily expressed by soluble solids content. The EU Regulation has laid down maturity requirements for *Vitis vinifera* L. cultivars (OJ L 157, 15.6.2011). The lowest allowed soluble solids content is 13 °Brix for seeded cultivars and 14 °Brix for seedless cultivars. In cool climate there are mainly cultivated grape hybrid cultivars which refractometric index is not regulated with this regulation. The aim of the present experiment was to investigate the accumulation dynamics and content of soluble solids from the beginning of veraison to harvest in table grapes with protected cultivation condition. The research was conducted with 3 black ('Osella', 'Kosmonavt', 'Mars'), 3 red ('Swenson Red', 'Somerset Seedless', 'Canadice') and 2 white ('Arkadia', 'Supaga') vine cultivars in 2013 and 2014. The results of the study indicated, that fruits of all table grape cultivars achieved the minimum content of soluble solids required for table grapes. Two years mean of soluble solids content varied among black, red and white grape cultivars respectively from 15.0 to 22.1 °Brix, from 15.6 to 22.5 °Brix and from 13.9 to 18.9 °Brix. The highest soluble solids content was observed in both years among black cultivars in Osella, among red cultivars in Somerset Seedless and among white in Supaga.

Key words: Brix, *Vitis* sp., hybrid cultivars.

INTRODUCTION

The grapes (*Vitis* sp.) are used for making wine, raisin and for fresh consumption, intended for table use. In Europe traditional grape growing region lies between 30° and 50° N (Gustafsson & Mårtensson, 2005). But in spite of the harsh climate grapes are also cultivatied in cool climate condition above 50° N. In Estonia grapes are cultivated in open field contitions and protected areas. Table grapes for commercial consumption are mainly grown on protected areas because in northen countries there is a problem with late spring and early autumn frost. Protected cultivation helps to decrease frost injuries and also helps to get earlier yield (the temperature is higher than on open field).

Grapes should not be harvested until mature, because they do not ripen after harvest (Nelson, 1985). Indicator of grape maturity is the sugar content, determined as the total soluble solids content in the berry juice and it is measured on a degree-Brix scale (Nelson, 1985). Growers mainly use it as an indicator of ripness (Muñoz-Robredo et al., 2011). For winegrowers it is the most practical parameter to look at because the sugar concentration determines the potential alchol content in the wine (Liu et al., 2006; Nogales-Bueno et al., 2014). Also table grape growers need to measure the sugar content because it is connected with grape technological maturity.

Soluble solids content depends on the cultivar and production area (Nelson, 1985). In the world there are marketing standards for table grapes cultivars grown from *V. vinifera* L. Overall rule is that table grape production cultivars are harvested with a lower level of soluble solids than wine grapes (Liu et al., 2006). In the EU minimum soluble solids content levels are given as 12 °Brix for the cultivars Alphonse Lavalleé, Cardinal and Victoria, 13 °Brix for all other seeded cultivars, and 14 °Brix for all seedless cultivars (OJ L-157 15/06/2011). These standarts are same in Afghanistan, but the minimum °Brix for the Indian markets is 16 (ETN 300, 2004). In the United States, in California and early production areas the minimum soluble solids content is 16.5 °Brix (Rees et al., 2012). According to the International Organisation Vine and Wine (OIV, 2008) tabel grapes with a Brix degree equal to or above 16 is considered as ripe. Recommended soluble solids content in red wine grapes are from 20 to 23°Brix (Schalkwyk & Archer, 2000).

Proceeding from the previous, we can set up a hypothesis: in cool climate conditions table grapes ripen and achieve desired level of soluble solids on protected area faster. The aim of the study was to determine the accumulation dynamics and content of soluble solids from the beginning of veraison to harvest in table grapes with protected cultivation condition.

MATERIALS AND METHODS

The research was conducted with 3 black ('Osella', 'Kosmonavt', 'Mars'), 3 red ('Swenson Red', 'Somerset Seedless', 'Canadice') and 2 white ('Arkadia', 'Supaga') vine hybrid cultivars in 2013 and 2014. The berry samples were collected from the protected cultivation area in West-Estonia at Lüüste village (58° 37′ 42″ N, 25° 8′ 17″ E). The grapevines were propagated *in vitro* and grown as own-rooted. The protected cultivation vineyard plastic tunnels 45 m in length, 8 m in width and 4 m in height were used. The protected area was covered with 0.18 thick UV stable low density polyethylene, at the end of April. Vines were planted in 2010, in 1.65 × 3.5 m spaces and trained in high double trunk trellis. White polypropylene fabric and spruce *(Picea*) branches were used as vine winter cover no additional heating system was used in plastic tunnels. The vine rows were oriented from north to south and ground covered with 0.04 mm thick black polyethylene plastic. The experimental area soil was sandy loam, pH_{KCl} 5.6 and 4.5% humus content. The soil P, K, Ca and Mg content was sufficient based on vine nutrients need and no additional fertilizers were used in experimental area. The experimental design was a randomized block with 3 replicates.

On the protected cultivation, in 2013 and 2014, the air temperatures were higher than open field and many years' means (Fig. 1). In 2013, average air temperatures on the protected cultivation and open field were respectively 21.6 °C and 17.8 °C in June, 21.1 °C and 17.5 °C in July, 18.9 °C and 16.6 °C in August, and 12.8 °C and 10.8 °C in September. In 2014, average air temperatures on the protected cultivation and open field were respectively 17.2 °C and 13.4 °C in June, 22.9 °C and 19.3 °C in July, 18.7 °C and 16.4 °C in August, and 13.1 °C and 11.1 °C in September. In June 2013 on the protected cultivation the air temperature were 4.4 °C higher and in July 1.7 °C lower than 2014. The average temperatures in Estonia in the period from 1971–2000 were respectively 15.1 °C in June, 16.9 °C in July, 15.6 °C in August and 10.4 °C in September. It appears that, in June 2014, in the open field the temperature was 1.7 °C lower than usual. Both years in August and September air temperature were similar among protected cultivation and among open field.

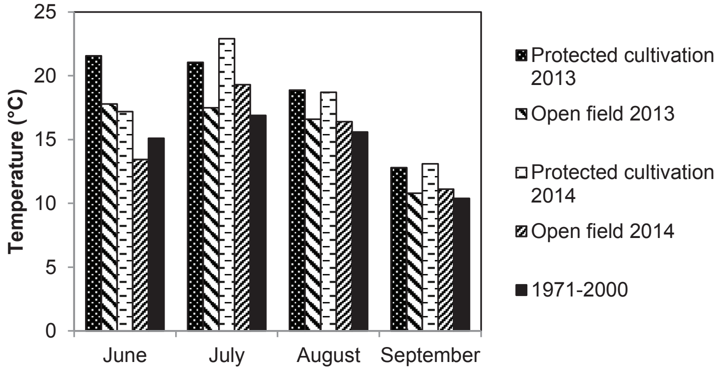


Figure 1. The mean air temperature in protected and open field in 2013, 2014 and the many years' means (1971–2000).

The soluble solids (SS, °Brix) measurements were carried out in 2013 from 08.08 to 20.09 and in 2014 from 7.08 to 12.09. The soluble solids content was measured from fresh berries by refractometer (Atago Pocket Refractometer Pal-1). For °Brix measurements, 30 grapes in 3 replications from the different parts of a cluster were picked and analysed.

The results of SS dynamics were tested by one-way analysis of variance. To evaluate significant influence, the least significant difference ($LSD_{0.05}$) was calculated. Different letters on figures mark significant differences at $P \leq 0.05$.

RESULTS AND DISCUSSION

In 2013 grapes SS content varied from the beginning of veraison to harvest from 8.7 to 22.6 °Brix and received at the minimum required level of maturity (EU standards) in different times (Fig. 2). At the beginning of August SS content varied from 8.7 to 16.3 °Brix. SS content changed in August among the cultivars 0.1 to 6.2 °Brix. SS content increased the most in cultivar Kosmonavt (10.5 to 16.7 °Brix) and least in cultivar Osella (14.1 to 14.2 °Brix). At the beginning of September SS content varied 13.0 to 20.5 °Brix and the last day of harvest 15.0 to 22.6 °Brix. SS content changed in September among cultivars 1.6 to 5.6 °Brix. SS content increased the most in cultivar Canadice (3.0 to 18.6 °Brix) and least in cultivar Mars (13.4 to 15.0 °Brix).

In 2014 grapes SS content varied from the beginning of veraison to harvest from 9.0 to 19.6 °Brix and received at the minimum required level of maturity (EU standards) in different times (Fig. 3). At the beginning of August SS content varied from 9.6 to 16.1 °Brix. SS content changed in August among the cultivars 1.5 to 6.1 °Brix. SS content increased the most in cultivar Supaga (10.6 to 16.7 °Brix) and least in cultivar Kosmonavt (16.1 to 17.6 °Brix). At the beginning of September SS content varied 12.7

to 18.1 °Brix and the last day of harvest 13.9 to 18.3 °Brix. SS content changed in September among cultivars 0.2 to 0.7 °Brix.

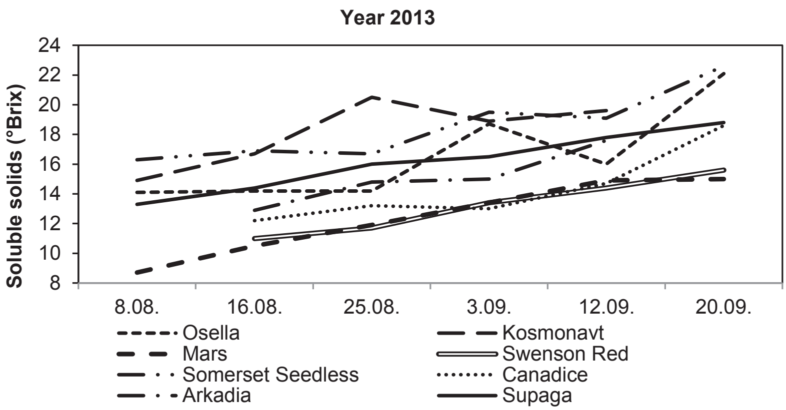


Figure 2. Table grape 'Osella', 'Kosmonavt', 'Mars', 'Swenson Red', 'Somerset Seedless', 'Candice', 'Arkadia' and 'Supaga' soluble solids content (°Brix) changes during the periods 08.08–20.09.2013.

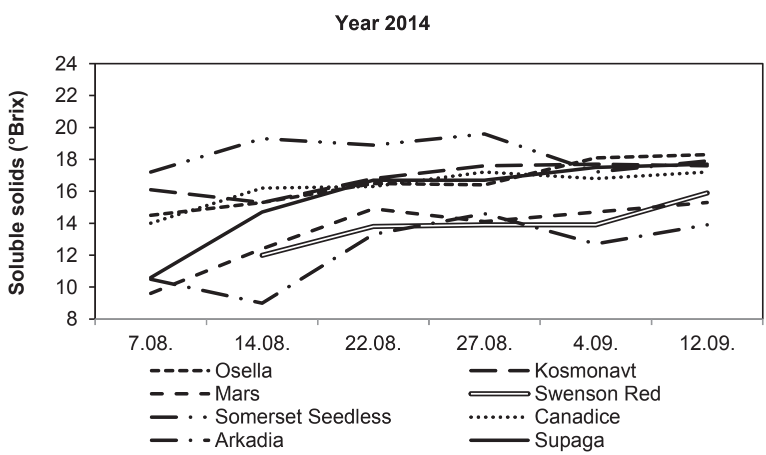


Figure 3. Table grape 'Osella', 'Kosmonavt', 'Mars', 'Swenson Red', 'Somerset Seedless', 'Candice', 'Arkadia' and 'Supaga' soluble solids content (°Brix) changes during the periods 7.08.–12.09.2014.

The rapid accumulation of sugars starts in grapes at the beginning of veraison and slows as maturity approaches (Bisson, 2001; Pedneault et al., 2013). It was also confirmed in our experiment. The beginning of veraison depends on the cultivar and growth conditions. The length of the veraison is 6 to 8 weeks (Plocher & Parke, 2008). For example in this experiment shorter ripening period has 'Osella' and 'Kosmonavt'. In 2013 SS content in the cultivars Kosmonavt and Osella drops with some evaluation terms. It could be caused by the fact that SS content is not valuable with bare eye and collecting samples we focus on berry color. Different phenolic compounds are responsible for the grape color. It is known maximum accumulation level of sugars and phenols do not coincide (Maujean et al., 1983). Because of that into the berry samples could get some berries with lower SS content. In this experiment cultivars Mars, Swenson Red and Arkadia started to ripen later than other cultivars in both year. Earliest cultivars were Somerset Seedless, Kosmonavt and Osella. Also their SS content were highest at the end of veraison, because of longer ripening period. In 2014 grapes started to ripen earlier, this is due to higher temperatures in July. Also because of higher temperatures SS content could be reached an optimum level faster in 2014 than 2013, as demonstrated by the SS content stability in the measuring period (variation was less than in 2013).

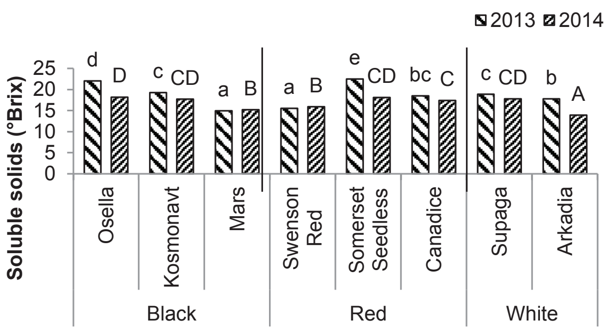


Figure 4. The grapes soluble solids (°Brix) content in the protected cultivation on cultivars Osella, Kosmonavt, Mars, Swenson Red, Somerset Seedless, Canadice, Supaga and Arkadia in 2013 and 2014. 2013 growing season cultivars effect PD%=0.9, 2014 growing season cultivars effect PD% = 0.8.

The content of SS ranged on the harvest from 15.0 to 22.6 °Brix in 2013 and 13.9 to 18.2 °Brix in 2014 (Fig. 4). In 2013 among black cultivars Osella had the highest value of SS (22.6 °Brix), but in both experimental year it was significantly lower in Mars (respectively 15.0 and 15.2 °Brix). Among red cultivars Somerset Seedless had the highest value of SS (22.1 °Brix) in 2013, but both experimental year it was lower in Swenson Red (respectively 15.6 and 15.9 °Brix). Among white cultivars Supaga had significantly higher values of SS in both years (respectively 18.9 and 17.8 °Brix). Year had a significant effect on table grapes SS content. In 2013 in several cultivars SS content was higher than 2014. In 2013 the harvest time was longer and because of that soluble solids accumulation period was also longer and °Brix values higher. Growing grapes on protected area we can extend the grape growing season, due to heat accumulation (Plocher & Parke, 2008). In Helsinki greenhouses accumulates 75 to 100% more heat than outdoor and because of that they can extend the growing season one month in the spring and in the autumn (Plocher & Parke, 2008). Fruits sugar concentration is higher in higher temperature conditions (Mira de de Orduńa, 2010) and it is also influenced by the genotype (Shiraishi et al., 2010). It was also confirmed in our study.

The EU Regulation provides maturity requirements for *V. vinifera* L. cultivars. The lowest allowed SS content is 13 °Brix for seeded cultivars and 14 °Brix for seedless cultivars. Compared to this requirement all our hybrid cultivars achieved these levels. But in the Nordic countries an important factor in the formation of taste are acids. Jayasena and Cameron (2008), on the Crimson Seedless cultivar, reported that the consumer acceptance increases with increasing SS content from 10 to 20 °Brix. In our experiment SS content was lowest in cultivars Mars, Swenson Red and Arkadia and highest in cultivars Osella, Kosmonavt and Somerset Seedless. Investigators of this experiment assessed better tasting cultivars Arkadia, Kosmonavt, Swenson Red and Somerset Seedless. This indicates that the taste is not determine only by SS content.

SS content differs among cultivars. For example optimum level for ‘Arkadia’ is find by Tairov National Research Centre for Viticulture and Winemaking 15 to 16 °Brix (Vinograd ‘Arkadia’, 2015), for ‘Kosmonavt’ is find by I.M. Filippenko 18.4 °Brix (Vinograd ‘Kosmonavt’, 2015), for ‘Mars’ find by J.N. Moore is 16 to 20 °Brix (Vinograd ‘Mars’, 2015) and for ‘Swenson Red’ find by E. Swenson is 17 to 18 °Brix (Myvinogradnik ‘Swenson Red’, 2015). In our experiment ‘Arkadia’ and ‘Kosmonavt’ reached that optimum level. ‘Mars’ and ‘Swenson Red’ SS content did not reach to optimum level, because they started to ripen later.

CONCLUSIONS

The study results indicates that soluble solids content in table grapes on protected area depends on the beginning of ripening on the cultivar and temperature. SS content was highest in cultivars Osella, Kosmonavt and Somerset Seedless. For growers it means that planning table grapes harvesting time they need to take into account temperature and cultivar ripening time.

ACKNOWLEDGMENTS. This research was supported by Estonian Science Foundation Grant No. 9363.

REFERENCES

Bisson, L. 2001. In search of optimal grape maturity. *Practical Winery Vineyard*, July/Aug 32–43.

ETN 003. 2004. Afghan Grapes: Grading for Quality. *http://afghanag.ucdavis.edu/a_horticulture/fruits-trees/grapes/fact-sheetsgrapes/ExtensionToolkitNotes3_GradingforQuality.pdf.pdf/view.* Accessed 21.01.2015

Gustafsson, J-G., Mårtensson, A. 2005: Review article: Potential for extending Scandinavian wine cultivation. *Acta Agriculturae Scandinavica, Section B – Soil & Plant Science* **55**, 82–97.

Jayasena, V., Cameron, I. 2008. °Brix/acid ratio as a predictor of consumer acceptability of Crimson Seedless table grapes. *Journal of Food Quality* **31**, 736–750.

Liu, H-F., Wu, B-H., Fan, P-G., Li, S-H., Li, L-S. 2006. Sugar and acid concentration in 98 grape cultivars analyzed by principal component analysis. *Science of Food and Agriculture* **86**, 1526–1536.
Maujean, A., Brun, O., Vesselle, G., Bureau, G., Boucher, J.M., Cousin, M., Feuillat, M. 1983. Investigations on grapevine maturation in the Champagne region: method of forecasting the harvesting date. *Vitis* **22**, 137–150.
Mira de Orduńa, R. 2010. Climate change associated effects on grape and wine quality and production. *Food Research International* **43**, 1844–1855.
Muñoz-Robredo, P., Robledo, P., Manriques, D., Molina, R., Defilippi, B.G. 2011. Characterization of sugars and organic acids in commercial varieties of table grapes. *Chilean Journal of Agricultural Research* **71**, 452–458.
Myvinogradnik 'Swenson Red'. *http://myvinogradnik.ru/sorta-vinograda/s/svenson-red/*. Accessed 30.01.2015.
Nelson, K.E. 1985. *Harvesting and handling California table grapes for market.* Second Edition, UCANR Publications, 67 pp.
Nogales-Bueno, J., Hernándes-Hierro, J.M., Rodrigues-Pulido, F.J. 2014. Determination of technological maturity of grapes and total phenolic compounds of grapes skins in red and white cultivars during ripening by near infrared hyperspectral image: A preliminary approach. *Food Chemistry* **152**, 586–591.
OIV. 2008. Resolution VITI 1/2008. OIV Standard on Minimum Maturity Requirements for Table Grapes. Organisation Internationale de la Vigne et du Vin, Paris. *ftp://ftp.fao.org/codex/meetings/cac/cac36/if36_05e.pdf.* Accessed 21.01.2015
OJ L-157 15/06/2011. Marketing standards for fresh fruits and vegetables. *http://exporthelp.europa.eu/update/requirements/ehir_eu13_02v001/eu/auxi/eu_mktfrveg_annex1b_r543_2011_grapes.pdf*. Accessed 10.12.2014
Pedneault, K., Dorais, M., Angers, P. 2013. Flavor of cold-hardy grapes: impact of berry maturity and environmental conditions. *Agricultural and Food Chemistry* **61**, 10418–10438.
Plocher, T., Parke, B. 2008. *Growing grapes and making wine in cool climate*. Second Edition, Northern Winework, 208 pp.
Rees, D., Farrell, G., Orchard, J. 2012. *Crop Post-Harvest: Science and Technology, Perishables.* John Wiley & Sons, 480 pp.
Schalkwyk, H., Archer, E. 2000. Determining optimum ripeness in wine grapes. *Wineland* **130**, 90–91.
Shiraishi, M., Fujishima, H., Chijiwa, H. 2010. Evaluation of table grape genetic resources for sugar, organic acid, and amino acid composition of berries. *Euphytica* **174**, 1–13.
Vinograd 'Arkadia'. *http://vinograd.info/sorta/stolovye/arkadiya.html*. Accessed 30.01.2015.
Vinograd 'Kosmonavt'. *http://vinograd.info/sorta/stolovye/kosmonavt.html*. Accessed 30.01.2015.
Vinograd 'Mars'. *http://vinograd.info/sorta/bessemyannye/mars.html*. Accessed 30.01.2015.

II

Maante-Kuljus, M., Vool, E., Mainla, L., Starast, M., Karp, K. 2019. Berry quality of hybrid grapevine (*Vitis*) cultivars grown in the field and in a polytunnel. *Agricultural and Food Science* 28(3): 137−144. doi:10.23986/afsci.76822

AGRICULTURAL AND FOOD SCIENCE

M. Maante-Kuljus et al. (2019) 28: 137–144

Berry quality of hybrid grapevine (*Vitis*) cultivars grown in the field and in a polytunnel

Mariana Maante-Kuljus, Ele Vool, Leila Mainla, Marge Starast and Kadri Karp

Department of Horticulture, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1a, EE51014 Tartu, Estonia

e-mail: mariana.maante-kuljus@emu.ee

The aim of this research was to determine the effect of vineyard location and cultivation system (polytunnel compared to field) on hybrid grapevine berry quality in cold climate conditions. The study was conducted with the hybrid grapevine cultivars 'Hasanski Sladki', 'Zilga' and 'Supaga'. Experimental vineyards were located at different sites in a polytunnel and in the field. Soluble solids content ranged from 13.8 to 25.4 °Brix. For all cultivars cultivation in the tunnel had a positive effect on soluble solids content for two experimental years out of three. Acid content was high, ranging from 1.04 to 1.76 g 100 g^{-1} FW. Growing 'Zilga' and 'Supaga' in the tunnel reduced titratable acid content every year but for 'Hasanski Sladki' in two years. Phenolic content ranged from 53 to 540 g 100 g^{-1} FW and anthocyanin from 30 to 162 mg 100 g^{-1} FW. Accumulation of phenols and anthocyanins were affected by location and cultivation methods. For 'Hasanski Sladki' in the tunnel, cultivation had a positive effect on phenolic content but for 'Zilga' not in every year.

Key words: soluble solids, titratable acid, juice pH, total anthocyanin, total phenolic

Introduction

In grapevine growing and wine making, work outcome depends on many different factors. French winemakers, who noticed differences in wines from different regions and vineyards, developed the concept of *terroir* which brings all factors together (Deloire et al. 2005, Edo-Roca et al. 2013, Tarricone et al. 2013). It involves interactions between cultivar, climate (macro-, meso-, topo- and microclimate), soil conditions (geology, pedology), vine water status, and human factors (viticultural practices) (van Leeuwen and Seguin 2006, Anesi 2015). According to Pereira et al. (2006), the grape metabolic profile is more affected by climatic characteristics (temperature and water balance) than soil conditions. The extent and timing of water stress is also important (Hunter and Deloire 2006). Increased anthocyanin concentration is related to fewer extreme temperature (>35 °C) days, lower vine water status and nitrogen level (Cheng et al. 2014).

In cold climate conditions, vine growers face several challenges: late spring and early autumn frosts, occasional cool summers, severe winters, and short growing seasons (Gustafsson and Mårtensson 2005). In traditional grapevine cultivation regions, the growing season is becoming warmer (Jones et al. 2005). Global warming is also observed in colder climate regions, such as Estonia: highly fluctuating winter temperatures and extreme weather conditions are more frequent; pests, diseases, and excess water are more abundant (Baseline report 2017). According to over ten years of monitoring in southernmost Finland, grape growing under existing weather conditions is possible (Karvonen 2014a). A study revealed that 'Zilga', crossbred in Latvia by Paul Sukatnieks, has acclimatized to Finnish growing conditions producing a soluble solids content of an average of 19 °Brix at harvest (Karvonen 2014b). In Sweden, mapping a sensor system for the Nordic Light Terroir character of wine has been developed in relation to climate change but retaining its fresh acidic/citrus nature (Nordmark et al. 2016). In Nordic countries, longer sunshine hours during the growing season can compensate for the shorter growing season and cooler summer. Cultivation of grapes in a polytunnel helps improve temperature conditions, enhancing earlier yield (Kamiloğlu et al. 2011). It can prolong the growing season, stimulate earlier vine bud break, and decrease vine injuries due to spring and autumn frosts. Cultivar selection is also important, and thus, *Vitis vinifera* and *V. labrusca*, *V. riparia, V. rupestris, V. aestivalis* (Lisek 2012), and *V. amurensis* (Gustafsson and Mårtensson 2005) hybrids are suitable for cold climate growing. Cultivation technologies (cane-girdling and cluster-berry thinning) can affect grape composition (Keskin et al. 2013). In Estonia, different techniques, such as pruning methods (Rätsep et al. 2014), defoliation (Maante et al. 2016), and cultivar effect on wine chemical composition (Pedastsaar et al. 2014) has been tested in the field. Technological maturity proved to be a problem because grape still had a high acid and a low sugar content. For phenolic maturity, which is important in red wine making, it was revealed that phenol and anthocyanin contents vary greatly.

Manuscript received November 2018

We can thus hypothesize that in a cold climate, growing interspecific hybrid grapevine cultivars in a polytunnel positively affects grape maturity. The aim of the study was to compare fruit technological and phenolic maturity parameters of the hybrid grape cultivars 'Hasanski Sladki', 'Zilga', and 'Supaga' grown in vineyards (in separate locations) with different cultivation systems (polytunnel and field).

Material and methods

Experimental sites and plant material

The experimental vineyards were established in 2007 with own-rooted plants of the hybrid wine grape cultivars: 'Hasanski Sladki' (*V. amurensis* × *V. labrusca* × *V. riparia* × *V. vinifera*), 'Zilga' (*V. amurensis* × *V. labrusca* × *V. vinifera*) and 'Supaga' (*V. vinifera* × *V. labrusca*). The experimental design was a randomized block with 4 replicates and 8 vines in each. In the field the (58° 21′ 27″ N, 26° 31′ 16″ E) vines were planted in 2×2 m spaces, trained on a low double trunk trellis, and spur pruned. The vines were not covered for winter. The polytunnel (58° 37′ 42″ N, 25° 8′ 17″ E) was 45 m long, 8 m in wide and 4 m in high. From the end of April until the beginning of November, the tunnel was covered with 0.18 mm thick UV stable low-density polyethylene. Vines were planted in 1.5×3.5 m spaces along the tunnel walls, trained in high double trunk trellis, and cane pruned. The tunnel did not have a foundation and therefore plants could get moisture from outside the tunnel. White polypropylene fabric was used as winter cover. The distance between the experimental sites was ca. 86 km. At both sites, the vine rows were oriented from north to south, the ground was covered with woven ground cover fabric and no additional irrigation was supplied. Leaves were removed from the cluster zone at the beginning of veraison (fruit coloration phase).

The soil in the field site was high-productivity sandy loam *Haplic luvisol* with sufficient drainage. That of the tunnel was a loam sandy *Gleyic albeluvisol* soil. Gleysol of the experimental site was a wet soil with prolonged surface and perched water. The road drainage ditch was located near the tunnel. Soil quality, evaluated on a 100-point scale, ranged from 45 to 50 in the field, and 30 to 34 points in the tunnel. The contents of available P, K, Ca and Mg were determined by the ammonium lactate method (Egnér et al. 1960). The following soil nutrient values in the field were determined: P and Mg – excessive, K – high, Ca – medium and pH_{KCl} was 5.4 (Table 1). P, K, Ca, and Mg values in the tunnel were medium and pH_{KCl} 5.6. No additional fertilizers were used in either experimental area.

Table 1. Nutrient content (mg kg^{-1}) of the soil in two experimental areas

Treatment	P	K	Mg	Ca	pH_{KCl}
Field	147	257	260	1670	5.4
Tunnel	22	170	98	830	5.6

Weather conditions

In the field, the frost-free period was 150, 140 and 149 days in 2013, 2014 and 2015, respectively (Table 2). The last spring frost was on 29 April, 7 May and 10 May in 2013, 2014 and 2015, respectively. The first autumn frost was on 26 September, 24 September and 6 October in 2013, 2014 and 2015, respectively. In 2013, the warmest month was June, in 2014 − July, and in 2015 – August. The sum of active temperatures (≥10 ℃) was 2490 ℃ in 2013, 2274 ℃ in 2014 and 2156 ℃ in 2015. The driest year was 2013 when precipitation from May to September was 269 mm, and the wettest year was 2014 (448 mm), compared to 2015 (309 mm), with a yearly mean of 330 mm. In 2013 and 2014, the driest month was September, and in 2015, it was August.

In the tunnel area, the frost-free period was shorter than in the field (136 days) in 2013 and 2014, but longer (163 days) in 2015. The last spring frost was on 28 April, 1 May and 25 April in 2013, 2014 and 2015, respectively. The first autumn frost was recorded in the tunnel earlier than in the field for two experimental years. In the tunnel, June was the warmest month in 2013, while in 2014 and 2015 it was July (Table 2). In all experimental years, mean temperature and daily temperature fluctuations were higher in the tunnel than in the field, with differences larger between maximum than minimum temperatures, especially in the spring months (May, June), in 2013 and 2015 (the difference was 10.0 to 19.1 °C).

In 2013, 'Supaga' had no yield due to cold damage during winter in the field, hence the biochemical parameters in Table 3 are marked with "–". For the analysis, berries were harvested in 2013−2015.

Table 2. Weather conditions in 2013−2015: monthly (mean, minimum and maximum) air temperature and total monthly precipitation sum compared to the mean of thirty years (1981–2010)

		Air temperature, °C							Precipitation, mm		
		Open field			Tunnel						
Year	Month	Min	Max	Mean	Min	Max	Mean	Years mean	Site 1	Site 2	Years mean
2013	May	3.7	28.1	15.5	4.2	38.1	20.4	10.4	73	45	42
	June	6.9	29.8	17.8	7.4	40.1	21.6	14.4	35	31	69
	July	8.4	28.7	17.5	8.3	37.5	21.1	17.4	59	43	72
	August	4.7	31.8	16.6	5.8	36.1	18.9	16.3	79	85	83
	September	−0.9	21.9	10.8	−2.3	27.5	12.8	11.5	23	55	64
2014	May	−2.1	30.9	12.3	−0.7	32.4	15.1	10.4	90	62	42
	June	2.5	27.5	13.7	11.7	29.9	17	14.4	134	75	69
	July	9.6	30.3	19.5	7.3	39.1	22.9	17.4	78	66	72
	August	4.5	29.7	16.8	5.8	34.4	18.7	16.3	126	102	83
	September	−0.3	23.4	12.5	−3.6	28.2	13.1	11.5	20	19	64
2015	May	−0.8	20.4	10.6	0.6	38.2	16.5	10.4	61	33	42
	June	3.7	24.6	14.6	4.2	43.7	19.6	14.4	66	58	69
	July	6.9	27.2	16.1	8.4	38.8	20.3	17.4	68	67	72
	August	3.6	30.7	17	5.4	40.3	19.3	16.3	47	25	83
	September	3.1	23.1	12.8	1.7	29.7	14.4	11.5	67	97	64

Site 1 = field; Site 2 = polytunnel. Temperature data were collected from an automatic weather station at the experimental sites. Sum of precipitation and many years mean monthly temperature and precipitation data were obtained from the Estonian Weather Station (www.ilmateenistus.ee) database

Technological maturity parameters

At harvest, the soluble solids content (SSC; °Brix) was determined using a digital refractometer (Atago Pocket Refractometer Pal-1). 30 grapes from each of the three replications from different parts of clusters were analyzed. Additionally, the weight of ten randomly selected vine clusters was determined in each replication, and the number of berries per cluster was recorded. Berry weight was calculated from the data obtained.

400 g of grapes from each of the three replications from the different parts of the cluster were analyzed. Titratable acid content (TAC) was determined by titration with 0.1 M NaOH solution to the endpoint of pH of 8.2 (Wrolstad et al. 2005) using a Mettler Toledo EasyPlus Titration titrator (with electrode DG 111-SC for endpoint detections). TAC was expressed in g of tartaric acid per 100 g fresh weight (FW). Soluble solids and titratable acidity ratio (SSC/TAC) was calculated. Grape juice pH was measured with pH/conductivity-meter (HD 2156.1, Delta OHM). Maturity index (MI) was calculated according to the formula: MI=°Brix × pH^2 (Coombe et al. 1980).

Phenolic maturity parameters

400 g of grapes from each of the three replications from the different parts of the cluster were analyzed. The total phenolic content (TPC) of grape skin (exocarp of fruit) was determined by the Folin-Ciocalteu phenol reagent method (Wrolstad et al. 2005) with a spectrophotometer (UVmini-1240 Shimdzu) at 765 nm. TPC was expressed in mg of gallic acid equivalent per 100 g of FW. Skin total anthocyanin content (ACC) was determined in cultivars ‘Hasanski Sladki’ and ‘Zilga’ using the pH-differential method. Absorbance was measured with a spectrophotometer (Uvmini-1240 Shimdzu) at 510 and 700 nm, in buffers with pH levels of 1.0 (HCl 0.1N) and 4.5 (citrate buffer). ACC was expressed in mg of cyanidin-3-glucoside equivalent per 100 g of FW.

Statistical analysis

The results were tested by one-way analysis of variance. To evaluate treatment effect within the cultivar, the least significant difference ($LSD_{0.05}$) was calculated, and different letters in the tables mark a significant difference at $p \le 0.05$. To evaluate main effects of two factors (cultivation system and experimental year), two-way analysis of variance was carried out and the difference was marked as non-significant (ns) or, using confidence levels, as significant at $p \le 0.05$*, 0.01** or 0.001***. Linear correlation coefficients were calculated between the variables (n = 18 'Hasanski Sladki' and 'Zilga', n = 15 'Supaga') with coefficient significance being $p \leq 0.05$*, and $p \leq 0.01$**. Relationship strength was estimated as $r \leq 0.3$ (weak), $0.3 \leq r \leq 0.7$ (moderate), and $r \geq 0.7$ (strong).

Results

Technological maturity

The SSC of 'Supaga' varied among treatments in all experimental years ranging from 13.8 to 19.5 °Brix (Table 3). There were significant differences between field and tunnel grown fruits. In 'Hasanski Sladki' and 'Zilga', SSC was from 15.1 to 25.4 °Brix and the difference was significant in two experimental years. In the tunnel, SSC increase ranged from 2 to 36% compared to the field. The main effect of treatment and year was significant at $p \leq 0.001$ for all cultivars.

Table 3. The effect of cultivation system on biochemical parameters and yield characteristics of 'Hasanski Sladki', 'Zilga' and 'Supaga' (2013–2015)

Year	Treatment	SSC	TAC	SSC/TAC	pH	MI	TPC	ACC	Berries/cluster, No	Cluster weight, g	Berry weight, g
'Hasanski Sladki'											
2013	Field	21.8 ^{b}	1.48 ^{a}	14.8 ^{b}	3.77 ^{a}	318 ^{a}	326 ^{b}	138 ^{b}	59 ^{a}	86 ^{a}	1.47 ^{a}
	Tunnel	25.4 ^{a}	1.09 ^{b}	23.3 ^{a}	3.68 ^{a}	344 ^{a}	480 ^{a}	162 ^{a}	39 ^{a}	71 ^{a}	1.80 ^{b}
2014	Field	19.1 ^{a}	1.61 ^{a}	11.9 ^{b}	3.18 ^{b}	194 ^{b}	254 ^{b}	30 ^{b}	29 ^{b}	27 ^{a}	0.94 ^{a}
	Tunnel	19.5 ^{a}	1.10 ^{b}	17.7 ^{a}	3.46 ^{a}	233 ^{a}	361 ^{a}	48 ^{a}	17 ^{a}	43 ^{a}	1.86 ^{b}
2015	Field	17.5 ^{b}	1.48 ^{a}	11.8 ^{b}	3.49 ^{b}	213 ^{b}	227 ^{b}	51 ^{b}	43 ^{a}	48 ^{a}	1.01 ^{a}
	Tunnel	21.8 ^{a}	1.43 ^{a}	14.7 ^{a}	3.60 ^{a}	282 ^{a}	274 ^{a}	79 ^{a}	41 ^{a}	73 ^{a}	1.70 ^{b}
Year		***	***	***	***	***	***	***	***	***	***
Treatment		***	***	***	**	***	***	***	**	ns	***
'Zilga'											
2013	Field	17.9 ^{b}	1.58 ^{a}	11.4 ^{b}	3.53 ^{b}	223 ^{b}	222 ^{b}	64 ^{b}	96 ^{b}	201 ^{a}	2.45 ^{a}
	Tunnel	24.1 ^{a}	1.19 ^{b}	20.2 ^{a}	3.60 ^{a}	312 ^{a}	540 ^{a}	112 ^{a}	55 ^{a}	189 ^{a}	3.84 ^{b}
2014	Field	15.1 ^{b}	1.47 ^{a}	10.3 ^{b}	3.08 ^{b}	143 ^{b}	344 ^{a}	32 ^{b}	41 ^{a}	48 ^{a}	2.72 ^{a}
	Tunnel	20.5 ^{a}	1.04 ^{b}	19.7 ^{a}	3.31 ^{a}	225 ^{a}	326 ^{b}	125 ^{a}	29 ^{a}	102 ^{b}	3.24 ^{b}
2015	Field	15.3 ^{a}	1.76 ^{a}	8.6 ^{b}	3.34 ^{a}	170 ^{b}	273 ^{a}	82 ^{a}	42 ^{a}	77 ^{a}	1.88 ^{a}
	Tunnel	17.6 ^{a}	1.58 ^{b}	11.1 ^{a}	3.35 ^{a}	198 ^{a}	256 ^{b}	79 ^{a}	58 ^{a}	209 ^{b}	3.23 ^{b}
Year		***	***	***	***	***	***	***	***	***	***
Treatment		***	***	***	***	***	***	***	*	***	***
'Supaga'											
2013	Field	–	–	–	–	–	–	×	–	–	–
	Tunnel	18.8	1.30	14.5	3.47	227	100	×	63	273	3.57
2014	Field	17.9 ^{b}	1.47 ^{a}	12.2 ^{b}	3.07 ^{b}	169 ^{a}	74 ^{b}	×	35 ^{a}	86 ^{a}	2.49 ^{a}
	Tunnel	19.5 ^{a}	1.11 ^{b}	16.0 ^{a}	3.36 ^{a}	200 ^{a}	104 ^{a}	×	59 ^{a}	167 ^{b}	3.06 ^{b}
2015	Field	13.8 ^{b}	1.67 ^{a}	8.2 ^{b}	3.15 ^{b}	137 ^{b}	228 ^{a}	×	68 ^{b}	121 ^{a}	1.82 ^{a}
	Tunnel	18.3 ^{a}	1.13 ^{b}	16.2 ^{a}	3.59 ^{a}	235 ^{a}	53 ^{b}	×	40 ^{a}	370 ^{b}	3.52 ^{b}
Year		***	**	**	***	ns	***	×	ns	***	ns
Treatment		***	***	***	***	***	***	×	ns	***	***

– = no yield; × = no results; SSC = soluble solids content (°Brix); TAC = titratable acid content (g 100 g^{-1} FW); SSC/TAC = soluble solids and titratable acid content ratio; TPC = total phenolic content (mg 100 g^{-1} FW); ACC = total anthocyanin content (mg 100 g^{-1} FW); MI = maturity index. Different letters in the same columns among cultivar and year according to the site mark significant differences of means at $p \leq 0.05$. Main effect of treatment and year: ns, *, **, *** = non-significant or significant influence at $p \leq 0.05$, $p \leq 0.01$ or 0.001, respectively.

The TAC in 'Zilga' and 'Supaga' varied from 1.04 to 1.76 g 100 g^{-1} FW and was influenced by treatment (Table 3). In 'Hasanski Sladki', TAC varied from 1.09 to 1.61 g 100 g^{-1} FW and the treatment had no effect only in 2013. In the tunnel, TAC decrease ranged from 3 to 32%. SSC/TAC was affected by the treatment and varied from 8.2 to 23.3. Thus in the field, SSC/TAC ratio decreased from 20 to 49%. For all cultivars, the main effect of treatment and year on TAC and SSC/TAC were significant.

The juice pH of 'Supaga' was significantly lower in the field and varied over the years from 3.07 to 3.59 (Table 3). For 'Zilga' and 'Hasanski Sladki' the same effect occurred in two years. In the field 'Zilga' pH varied between 3.08 to 3.53 and 'Hasanski Sladki' 3.18 to 3.77. In the tunnel 'Zilga' pH was between 3.31 to 3.60 and 'Hasanski Sladki' 3.46 to 3.68. For all cultivars the main effect of treatment and year was significant.

The MI in 'Zilga' was significantly higher in the tunnel for all experimental years, whereas in 'Hasanski Sladki' and 'Supaga' the difference was not significant in one year (Table 3).The main effect of treatment and year was significant at $p \leq 0.001$ for 'Hasanski Sladki' and 'Zilga', but for 'Supaga' only treatment had a significant effect.

Phenolic maturity

The TPC differed significantly with treatment: in the tunnel, TPC of 'Hasanski Sladki' increased in all experimental years, while for 'Zilga' only in one year (Table 3). For 'Supaga' the TPC was positive in one year but negative in the other year. The ACC in 'Hasanski Sladki' grapes differed between field and tunnel in all experimental years and was significantly higher in the tunnel. In 'Zilga', the effect of treatment emerged in 2013 and 2014. The main effects of treatment and year on TPC and ACC were significant at $p \leq 0.001$.

Yield characteristics

The number of berries per cluster varied with cultivar: 'Hasanski Sladki' 17 to 59, 'Zilga' 29 to 96 and 'Supaga' 35 to 68 (Table 3). This variability was caused by year and all cultivars had a location effect in one year. In the tunnel 'Zilga' and 'Supaga' had significantly heavier cluster in 2014 and 2015. For all cultivars effect of year was significant. There was a significant effect of the treatment and experimental year on berry weight. In the tunnel, the grapes of all cultivars were heavier than those from the field.

Correlations

SSC and SSC/TAC in all cultivars had a strong positive correlation with berry weight (Table 4). TAC, on the other hand, had a strong negative correlation with berry weight. The juice pH in 'Hasanski Sladki' correlated positively with all berry parameters, in 'Zilga' − with berries per cluster and cluster weight (moderate and strong, respectively) and in 'Supaga' − with cluster weight and berry weight (both strong). In all cultivars, MI correlated positively with cluster weight and berry weight for 'Hasanski Sladki' and 'Supaga' both strong, for 'Zilga' – cluster weight moderate and berry weight strong. TPC correlated with berry weight – 'Hasanski Sladki' had a strong positive correlation, 'Zilga' had a moderately positive correlation, but 'Supaga' had a strong negative correlation. In 'Hasanski Sladki', ACC correlated positively with all berry parameters: for berries per cluster and berry weight moderate and for cluster weight strong. In 'Zilga', ACC had a positive correlation only with berry weight (moderate).

Table 4. The correlation coefficients between cluster characteristics and berry biochemical parameters of grapevine cultivars 'Hasanski Sladki', 'Zilga' and 'Supaga'

Parameters	SSC	TAC	SSC/TAC	pH	MI	TPC	ACC
'Hasanski Sladki'							
Berries/cluster	0.265 ns	0.324 ns	−0.076 ns	0.617 **	0.462 ns	−0.067 ns	0.524 *
Cluster weight, g	0.629 **	−0.136 ns	0.382 ns	0.875 **	0.801 **	0.341 ns	0.739 **
Berry weight, g	0.668 **	−0.773 **	0.797 **	0.542 *	0.660 **	0.733 **	0.503 *
'Zilga'							
Berries/cluster	0.069ns	0.337 ns	−0.187 ns	0.588 *	0.257 ns	−0.273 ns	−0.070 ns
Cluster weight, g	0.465ns	0.023 ns	0.175 ns	0.743 **	0.599 **	−0.073 ns	0.197 ns
Berry weight, g	0.774**	−0.699 **	0.760 **	0.305 ns	0.668 **	0.509 *	0.498 *
'Supaga'							
Berries/cluster	−0.199 ns	−0.040 ns	−0.027 ns	0.102 ns	−0.040 ns	0.388 ns	×
Cluster weight, g	0.467 ns	−0.702 **	0.690 **	0.888 **	0.799 **	−0.452 ns	×
Berry weight, g	0.857 **	−0.835 **	0.891 **	0.858 **	0.975 **	−0.757 **	×

× = no results; SSC = soluble solids content (°Brix); TAC = titratable acid content (g 100 g^{-1} FW); SSC/TAC = soluble solids and titratable acid content ratio; TPC = total phenolic content (mg 100 g^{-1} FW); ACC = total anthocyanin content (mg 100 g^{-1} FW); MI = maturity index; ns = correlation coefficients between variables with the significance coefficient being non-significant, significant at $p \leq 0.05$* or 0.01**

Discussion

In wine making, recommended values for berry technological maturity are used (van Schalkwyk and Archer 2000). The recommended °Brix in grapes for red wines is from 20.5 to 23.5, and for white wines – from 19.5 to 23. Hybrid cultivars in the current experiment were *labrusca* type, and for this kind of grape, the optimum can range from 17 to 18 °Brix (Plocher and Parke 2008). 'Hasanski Sladki' achieved the optimum (17–18 °Brix) level or above it in both experimental sites in all three years. In all experimental years, 'Zilga' and 'Supaga' reached the optimum level of °Brix in the tunnel, but not in all years in the field. The recommended TAC value should range from 0.65 to 0.75 g 100 g^{-1} FW for red table wines, and from 0.7 to 0.8 g 100 g^{-1} FW for white table wines (van Schalkwyk and Archer 2000). In the tunnel, TAC was lower, but it was still significantly higher than the recommended level in all experimental cultivars. The recommended juice pH for white table wine is between 3.0 and 3.3, and for red table wine between 3.2 and 3.4. The recommended level was achieved by 'Zilga' in tunnel and field in 2014 and 2015, by 'Supaga' in tunnel and field in 2014 and by 'Hasanski Sladki' in the field only and only in 2014.

The growing of grapes in the tunnel significantly affected their technological maturity parameters: for all cultivars in most cases, SSC and juice pH were significantly higher and TAC lower in the tunnel. According to Tarara et al. (2008), higher temperatures during ripening decrease TAC and increase juice pH. Higher temperatures could have also affected TAC and juice pH in the current study. In test sites, there were significant differences in air temperatures. In the tunnel, the mean temperature in May was 4.9, 2.8 and 5.9 °C higher (2013, 2014 and 2015, respectively) than in the field, and caused earlier growth of vines. In grapes, acids are formed during the first growth period (Kennedy 2002). Our results indicated that higher temperatures in the tunnel (3 to 5 °C in June and 3 to 4 °C in July) reduced the concentration of acid. The temperature was also higher during the period of *veraison* in August and September. During that period, higher temperature and greater daily temperature fluctuation influenced soluble solids and caused the reduction of acid levels. Tunnel monthly minimum and maximum temperatures differed almost by 30 °C. The recommended MI level ranges from 200 to 270 (van Schalkwyk and Archer 2000). In the tunnel, MI reached the recommended level, and the results were influenced by higher SSC and juice pH values. Correlation results indicate that, cluster characteristics had a relationship with berry composition. All maturity parameters had a correlation with berry weight. In heavier berries, the SSC was higher and acid content lower. In cold climate conditions, the effect of vineyard location is important, and therefore, growers need to choose the warmest possible growing sites or use other methods, like tunnel cultivation.

There was a significant effect of cultivation system and location on phenolic maturity. Differences in experimental results were affected by vineyard location conditions, climate factors and cultivar properties. The soil of the two experimental areas differed – that in the field was more fertile than that in the tunnel. The impact of site on the test results was related to the soil and is consistent with previous experiments. Lower phenolic content of grapes is caused by more fertile vineyard soil (de Andrés-de Prado et al. 2007), and a decrease in polyphenol content with a higher nitrogen supply (Heimler et al. 2017). The growth habit of the experimental cultivars differed − 'Hasanski Sladki' is more vigorous than 'Zilga'. More vigorous growth and a longer plant growth period affects maturation and TPC and ACC values. Accumulation of phenolic compounds in grape skin is influenced by environmental factors (Soubeyrand et al. 2014), depends on cultivar (Katalini et al. 2010, Samoticha et al. 2017) and is highly influenced by *terroir* (Tarko et al. 2010). In the tunnel, the temperature was higher during the period of *veraison* in August (1.9 to 2.3 °C) and September (0.6 to 2 °C) than in the field. During this period, higher temperature and greater night and day temperature fluctuation influenced total phenol and anthocyanin accumulation. In the tunnel, monthly maximum temperatures were always higher than in the field. Day and night temperature fluctuation was also greater. For example, in 2015, in August, day and night temperature differences varied from 17.0 to 27.0 °C in the tunnel, but from 4.3 to 18.8 °C in the field.

Correlation analyses indicate that cluster characteristics had a relationship with phenolic maturity. For all cultivars TPC had a relationship with berry weight. 'Hasanski Sladki' ACC had positive correlation with all cluster characteristics, but for 'Zilga' only with berry weight. The effect of temperatures and sun exposure on anthocyanins depended on cluster distinctness − in thinner clusters, grapes are better exposed to light than in tighter clusters. 'Hasanski Sladki' clusters are thinner and, therefore the grapes are more exposed to sun and temperature fluctuation impact may have been bigger than in 'Zilga' with its denser and larger clusters. Cluster and berry weights can be affected by growing conditions. The differences in growing conditions between the tunnel and the field could have caused variation in compound bud vitality: whether shoots developed from a larger central primary bud, from smaller secondary buds, or from both at the same time. In the field, the growing season is shorter, and therefore, the vine primary bud may remain less cold hardy. When the primary bud is damaged, the smaller secondary or tertiary bud will break, which will greatly affect yield formation.

Conclusion

Until now, in Estonia, table grape cultivars have been cultivated in tunnels and wine cultivars in the field. Our results support the hypothesis that in cold northern climate conditions, different vineyard cultivation systems affect the maturity of hybrid grapevines. In the tunnel, grapes had better technological maturity parameters – SSC increased and TAC decreased. On phenolic maturity, the treatment effect was significant and depended on the year and cultivar properties. Thus, for technological maturity enhancement, cultivation in polytunnels is recommended. The results confirmed the need for further research on special cultivars and cultivation methods in Estonia to explore the quality potential of grapes for winemaking.

Acknowledgment

This research was supported by Estonian Science Foundation Grant No. 9363, "Targeted Funding" project SF0170057s09 and Institutional Research Funding project IUT36-2.

References

Anesi, A., Stocchero, M., Dal Santo, S., Commisso, M., Zenoni, S., Ceoldo, S., Tornielli, G.B., Siebert, T.E., Herderich, M., Pezzotti, M. & Guzzo, F. 2015. Towards a scientific interpretation of the terroir concept: plasticity of the grape berry metabolome. BMC Plant Biology 15: 191. https://doi.org/10.1186/s12870-015-0584-4

Baseline report 2017. Baseline report for the 4 main EU Climate Risk Regions 2017. https://agriadapt.eu/documents/. Accessed 1 March 2018.

Cheng, G., He, Y.N., Yue, T.X., Wang, J. & Zhang, Z.W. 2014. Effects of Climatic Conditions and Soil Properties on Cabernet Sauvignon Berry Growth and Anthocyanin Profiles. Molecules 19: 13683–13703. https://doi.org/10.3390/molecules190913683

Coombe, B.G., Dundon, R.J. & Short, A.W.S. 1980. Indices of sugar-acidity as ripeness criteria for wine grapes. Journal of the Science of Food and Agriculture 3: 495–502. https://doi.org/10.1002/jsfa.2740310512

de Andrés-de Prado, R., Yuste-Rojas, M., Sort, X., Andrés-Lacueva, C., Torres, M. & Lamuela-Raventós, R.M. 2007. Effect of soil type on wines produced from *Vitis vinifera* L. cv. Grenache in commercial vineyards. Journal of Agricultural and Food Chemistry 55: 779–786. https://doi.org/10.1021/jf062446q

Deloire, A., Vaudour, M.E., Carey, V., Bonnardot, V. & van Leeuwen, V. 2005. Grapevine responses to terroir: a global approach. Journal international des sciences de la vigne et du vin 39: 149–162. https://doi.org/10.20870/oeno-one.2005.39.4.888

Edo-Roca, M., Nadal, M. & Lampreave, M. 2013. How terroir affects bunch uniformity, ripening and berry composition in *Vitis vinifera* cvs. Carignan and Grenache. Journal international des sciences de la vigne et du vin 47: 1–20. https://doi.org/10.20870/oeno-one.2013.47.1.1533

Egnér, H., Riehm, H. & Domingo, W.R. 1960. Untersuchungen uber die chemische Bodenanalyse als Grundlage fur die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung. Kungliga Lantbrukshögskolans Annaler 26: 199–215.

Gustafsson, J.-G. & Mårtensson, A. 2005. Potential for extending Scandinavian wine cultivation. Acta Agriculturae Scandinavica, Section B - Soil & Plant Science 55: 82–97. https://doi.org/10.1080/09064710510029097

Heimler, D., Romani, A. & Leri, F. 2017. Plant polyphenol content, soil fertilization and agricultural management: a review. European Food Research and Technology, 243: 1107–1115. https://doi.org/10.1007/s00217-016-2826-6

Hunter, J.J. & Deloire, A. 2006. Terroir and vine water relation effects on grape ripening and wine quality of Syrah/R99. Proceedings of the 4th International terroir congress, 3-7 July in Bordeaux and Montpellier, France. p. 110−116.

Jones, G.V., White, M.A., Cooper, O.R. & Storchmann, K. 2005. Climate change and global wine quality. Climate Change 73: 319–343. https://doi.org/10.1007/s10584-005-4704-2

Kamiloğlu, Ö., Polat, A.A. & Durgaç, C. 2011. Comparison of open field and protected cultivation of five early table grape cultivars under Mediterranean conditions. Turkish Journal of Agriculture and Forestry 35:491–499. https://doi.org/10.3906/tar-1002-718

Karvonen, J. 2014a. The annual growth cycle of grapevines in Southern Finland. Vitis 54: 175–180.

Karvonen, J. 2014b. *Vitis* cv. Zilga is a vine for the northern temperate climate - Short communication. Horticultural Science (Prague) 41: 147–151. https://doi.org/10.17221/17/2014-HORTSCI

Katalini, V., Moina, S.S., Skroza, D., Generali, I., Abramovi, H., Milo, M., Ljubenkov, I., Piskernik, S., Pezo, I., Terpinc, P. & Boban, M. 2010. Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 *Vitis vinifera* varieties grown in Dalmatia (Croatia). Food Chemistry 119: 715–723. https://doi.org/10.1016/j.foodchem.2009.07.019

Kennedy, J. 2002. Understanding grape berry development. Practical Winery & Vineyard 1–5.

Keskin, N., İşçi, B. & Gökbayrak, Z. 2013. Effect of cane-girdling and cluster and berry thinning on berry organic acids of four *Vitis vinifera* L table grape cultivar. Acta Scientiarum Polonorum Hortorum Cultus 12: 115–125.

Lisek, J. 2012. Winter frost injury of buds on one-year-old grapevine shoots of Vitis vinifera cultivars and interspecific hybrids in Poland. Folia Horticulturae 24: 97–103. https://doi.org/10.2478/v10245-012-0010-4

Maante, M., Vool, E. & Karp, K. 2016. Effect of defoliation on grape maturity parameters. Sodininkyste ir Darzininkyste 35: 21−35.

Nordmark, L., Lindén, J., Skjöldebrand, C. & Hansson, H. 2016. The Nordic Light Terroir. Acta horticulturae 1115: 189–194. https://doi.org/10.17660/ActaHortic.2016.1115.28

Pedastsaar, P., Vaher, M., Kulp, M., Kaljurand, M., Karp, K., Raal, A., Karathanos, V. & Püssa, T. 2014. Chemical composition of red wines made from hybrid grape and common grape (*Vitis vinifera* L.) cultivars. Proceedings of the Estonian Academy of Sciences 63: 444–453. https://doi.org/10.3176/proc.2014.4.10

Pereira, G.E., Gaudillere, J.P., van Leeuwen, C., Hilbert, G., Maucourt, M. & Deborde, C. 2006. H1-NMR metabolite fingerprints of grape berry: Comparison of vintage and soil effects in Bordeaux grapevine growing areas. Analytica Chimica Acta 563: 346–352. https://doi.org/10.1016/j.aca.2005.11.007

Plocher, T. & Parke, B. 2008. Northern Wineworks: growing grapes and making wine in cold climates, 2th Ed. Northern Winework Inc., Hugo, Minnesota. 214 p.

Rätsep, R., Karp, K., Vool, E. & Tõnutare, T. 2014. Effect of pruning time and method on hybrid grapevine (*Vitis* sp.) 'Hasanski Sladki' berry maturity in a cool climate conditions. Acta Scientiarum Polonorum Hortorum Cultus 13: 99−112.

Samoticha, J., Wojdyło, A. & Golis, T. 2017. Phenolic composition, physicochemical properties and antioxidant activity of interspecific hybrids of grapes growing in Poland. Food Chemistry 215: 263–273. https://doi.org/10.1016/j.foodchem.2016.07.147

Soubeyrand, E., Basteau, C., Hilbert, G., van Leeuwen, C., Delrot, S. & Gomès, E. 2014. Nitrogen supply affects anthocyanin biosynthetic and regulatory genes in grapevine cv. Cabernet-Sauvignon berries. Phytochemistry 103: 38–49. https://doi.org/10.1016/j.phytochem.2014.03.024

Tarara, M.J., Lee, J., Spayd, E.S. & Scagel, F.C. 2008. Berry temperature and Solar Radiation alter acylation, propotion, and concentration of anthocyanin in Merlot grapes. American Journal of Enology and Viticulture 59: 235–247.

Tarko, T., Duda-Chodak, A., Sroka, P., Satora, P. & Jurasz, E. 2010. Polish wines: characteristics of cool-climate wines. Journal of Food Composition and Analysis 23: 463–468. https://doi.org/10.1016/j.jfca.2010.01.009

Tarricone, L., Masi, G., Amendolagine, A., Di Gennaro, D., Suriano, S., Savino, M., Bavaresco, L. & Gardiman, M. 2013. Terroir influence on viticultural and oenological characteristics of *Vitis vinifera* cv. Negro Amaro. Proceedings of the 36th World Congress of vine and wine, in 2-7 June in Bucharest, Romania. http://hdl.handle.net/10807/61704

van Leeuwen, C. & Seguin, G. 2006. The concept of terroir in viticulture. Journal of Wine Research 17: 1–10. https://doi.org/10.1080/09571260600633135

van Schalkwyk, H. & Archer, E. 2000. Determining optimum ripeness in wine grapes. Wineland 130: 90–91.

Wrolstad, R.E., Acree, T.E., Decker, E.A., Penner, M.H., Reid, D.S., Schwartz, S.J., Shoemaker, C.F., Denise, M.S. & Sporns, P. 2005. Handbook of Food Analytical Chemistry. Pigments, colorants, flavors, texture, and bioactive food components. New Jersey: John Wiley & Sons. 624 p. https://doi.org/10.1002/0471709085

III

Maante-Kuljus, M., Rätsep, R., Mainla, L., Moor, U., Starast, M., Põldma, P., Karp, K. 2019. Technological maturity of hybrid vine (*Vitis*) fruits under Estonian climate conditions. *Acta Agriculturæ Scandinavica, Section B - Soil & Plant Science* 69(8): 706−714. doi:10.1080/09064710.2019.1641547

Acta Agriculturae Scandinavica, Section B — Soil & Plant Science

ISSN: 0906-4710 (Print) 1651-1913 (Online) Journal homepage: https://www.tandfonline.com/loi/sagb20

Technological maturity of hybrid vine (*Vitis*) fruits under Estonian climate conditions

Mariana Maante-Kuljus, Reelika Rätsep, Leila Mainla, Ulvi Moor, Marge Starast, Priit Põldma & Kadri Karp

To cite this article: Mariana Maante-Kuljus, Reelika Rätsep, Leila Mainla, Ulvi Moor, Marge Starast, Priit Põldma & Kadri Karp (2019) Technological maturity of hybrid vine (*Vitis*) fruits under Estonian climate conditions, Acta Agriculturae Scandinavica, Section B — Soil & Plant Science, 69:8, 706-714, DOI: 10.1080/09064710.2019.1641547

To link to this article: https://doi.org/10.1080/09064710.2019.1641547

Published online: 12 Jul 2019.

ACTA AGRICULTURAE SCANDINAVICA, SECTION B — SOIL & PLANT SCIENCE
2019, VOL. 69, NO. 8, 706–714
https://doi.org/10.1080/09064710.2019.1641547

Taylor & Francis
Taylor & Francis Group

Check for updates

Technological maturity of hybrid vine (*Vitis*) fruits under Estonian climate conditions

Mariana Maante-Kuljus[a], Reelika Rätsep [b], Leila Mainla[a], Ulvi Moor [a], Marge Starast[a], Priit Põldma [a] and Kadri Karp[a]

[a]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia; [b]Institute of Agricultural and Environmental Sciences, Polli Horticultural Research Centre, Polli, Estonia

ABSTRACT

Vine growing is gaining popularity around the Baltic Sea and the production of commercial wine is also increasing in Estonia. The aims of the experiment were: evaluate the suitability of different grape cultivars for growing in Estonian climate according to Heliothermal Index; determine the wine grapes technological maturity of 'Hasansky Sladky', 'Zilga' and 'Rondo' over 10 years; determine the effect of vineyard locations with different cultivation systems (polyethylene tunnel and field) on wine grapes technological maturity and acids composition of 'Rondo'. In the field conditions, the soluble solids content ranged from 12.0 to 21.2 °Brix. Titratable acids content ranged from 6.5 to 22.7 g L^{-1} and the pH was between 2.8 and 3.9. Growing 'Rondo' in tunnel increased soluble solids and decreased total acids content. Tartaric acid content ranged from 3.6 to 5.5 g L^{-1} in field and from 3.7 to 4.3 g L^{-1} in the tunnel. In the field, malic acid in grapes was significantly higher (5.2–7.8 g L^{-1}) when compared to grapes grown under tunnel conditions (1.9–3.6 g L^{-1}). Estonia is classified as a region with very cool climate for vine cultivation. The latter indicates to the existence of the heliothermal potential for growing early cultivars.

ARTICLE HISTORY
Received 3 May 2019
Accepted 4 July 2019

KEYWORDS
Heliothermal Index; malic acid; pH; soluble solids; tartaric acid; titratable acids

Introduction

Whether or not grape growing is possible depends on the climate of the growing area. In vine growing Heliothermal Index (HI) of Huglin (1978) provides information about the heliothermal potential. It ranges from ≤1500°C (very cool) to >3000°C (very warm) (Tonietto and Carbonneau 2004). This gives a measure on the suitability of a region for growing different grape cultivars based on the daily mean and maximum temperatures of the region, and on a factor denoted as the length of day coefficient, which depends on the geographic latitude of the region (and thus the average length of the days during the growing season). It has been used along with other temperature indices to define a region's potential for viticulture (Jones et al. 2010). Similarly to Finland (neighbour country) which belongs to the very cool area according to HI (Karvonen 2017), in Estonia, the location and temperatures are also important for the selection of cultivars.

The correct harvest time determines the quality of wine grapes, which is decided by the contents of the primary and secondary metabolites. Technological maturity is based on pulp sugar and acids contents and pH value. The amount of sugars in grapes influences potential alcohol content in wines, and wine sensory attributes as well (Heymann et al. 2013). The experiment with 'Cabernet Sauvignon' wines, made from grapes with lower sugar content turned more sour and had more fresh vegetative flavour, while the wines made from grapes with high soluble solids content were more strong and bitter taste, and in some cases had more intensive fruit flavour and sweetness. *Labrusca* type hybrid grapes can be harvested when soluble solids content ranges from 17 to 18 °Brix and before the intensive *labrusca* flavour develops (Plocher and Parke 2008). Table grapes are considered ripe when Brix° is 16 (OIV resolution Viti 1/2008). The titratable acidity and pH affects wine quality, colour, and taste. According to Tarara et al. (2008), higher temperatures during ripening decrease acids content and increase juice pH. In very cool climate conditions, there is a problem with reaching the desired maturity; more precisely, there is a problem with low sugar and high titratable acids content (Gustafsson and Mårtensson 2005). Grapes' technological maturity also depends on canopy management techniques

CONTACT Mariana Maante-Kuljus mariana.maante-kuljus@emu.ee Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, 51006 Tartu, Estonia

such as pre-*veraison* suckering removal, shoot positioning, topping, leaf thinning and leaf removal adjacent to berry clusters at the beginning of *veraison* (Plocher and Parke 2008). Leaf thinning at pea-size fruit growth stage increased glucose and fructose concentration and decreased malic acid concentration in grapes (Hunter et al. 2004). Leaf removal at *veraison* generally increased sugar and decreased titratable acids contents (Baiano et al. 2015). In Estonia, different techniques have been tested to regulate grape ripening such as spur and cane pruning methods (Rätsep et al. 2014) and leaf removal at different fruit development stages (Maante et al. 2016). Moreover, reaching the proper technological maturity has been a problem in the open field conditions and therefore growing grapes in the high polyethylene tunnel is gaining the attention of the producers. Hybrid grape cultivars such as 'Hasansky Sladky', 'Zilga' and 'Rondo' are common for Estonian vine growers, but scientific knowledge on the proportion of individual acids in such cultivars is insufficient for winemaking.

Tartaric and malic acids are the most abundant organic acids in grapes and generally account for 62–92% of all organic acids (Kliewer 1966). The acids are synthesised actively up to *veraison*, after which tartaric acid content stays fairly constant (Ruffner 1982; Volschenk et al. 2006). Malic acid content starts to decrease due to its metabolism through different pathways (Sweetman et al. 2009). The rate of degradation of malic acid depends on the temperature of growing environment: higher temperatures at *veraison* and during ripening stages reduce malic acid content (Sweetman et al. 2014; Kizildeniz et al. 2018). The level of malic acid is usually higher in cool climate regions and lower in warmer regions (Volschenk et al. 2006; Conde et al. 2007). The tartaric acid is not as rapidly lost at high temperatures as malic acid (Kliewer 1971). Also, leaf thinning at pea-size fruit development stage reduced malic acid concentration and influenced tartaric to malic acids ratio (Hunter et al. 2004). Higher malic acid content causes a sour/green/sharp taste in wine (Volschenk et al. 2006). A high ratio of tartaric to malic acid improves the stability of wine (Liu et al. 2007). Based on the previously mentioned aspects, more precise information on the contents of tartaric and malic acid and their ratio in wine grapes will lead to the understanding of the necessity of malolactic fermentation and selection of appropriate yeast strains for winemaking process.

In comparison to open field conditions, cultivating vines in a high polyethylene tunnel could improve the technological maturity parameters of wine grapes: reduce total acids, including malic acid content. The first aim was to analyse the suitability of Estonian climate for grape growing, according to Heliothermal Index and its dynamics. The second aim of the study was to determine the variability of technological maturity among the experimental years in hybrid grape cultivars 'Hasansky Sladky', 'Zilga' and 'Rondo'. The third aim was to determine the effect of vineyard locations with different cultivation systems (high polyethylene tunnel and open field) on fruit technological maturity and acids composition of 'Rondo'.

Materials and methods

Experimental sites and plant material

The open field (58° 21′ 27″ N, 26° 31′ 16″ E) vineyard was established in 2007 with own-rooted plants of three hybrid grape cultivars: 'Hasansky Sladky', 'Zilga' and 'Rondo'. Vines were planted in 2×2 m spaces, trained in low double trunk trellis and spur pruning was used. In the impact assessment of the year, yield data were collected from 2009 to 2018. In 2010 'Zilga' had spring frost damage on flowers and in 2013 and 2014 'Rondo' had winter cold damage of canes (Table 2; '×' marks years without harvest of these cultivars). With cultivar 'Rondo' from 2016 to 2018, there were two variants: open field and high polyethylene tunnel cultivating systems in two locations. The experimental design was a randomised block with 4 replicates and 8 vines in each. High polyethylene tunnel (58° 17′ 1″ N, 26° 33′ 41″ E) was 28 m in length, 7.6 m in width and 4.6 m in height, covered with 0.18 mm thick UV stable low-density polyethylene. Vines were planted in 1.6×2 m spaces, trained in low double trunk trellis and cane pruning was used. White polypropylene fabric was used as a winter cover. The tunnel does not have a foundation and therefore plants get moisture from outside the tunnel. The distance between the experimental areas was ca. 8.5 km. The different planting and canopy management in open field and tunnel conditions are according to the specificity of growing site. In both sites, the vine rows were oriented from north to south, the ground was covered with woven ground cover fabric, and no additional irrigation system was used. Furthermore, at the beginning of *veraison*, leaves were removed from the cluster zone.

The soil in both experimental areas was high-productivity sandy loam *Haplic luvisol*. Soils were sufficiently drained and soil fertility was 45–50 points in 100-point scale. The open field soil nutrient content was determined: P and Mg – excessive, K – high, Ca – medium and pH_{KCl} was 5.4. P, K, Ca and Mg values in the tunnel were high and pH_{KCl} was 5.4. No additional fertilisers were used in either experimental areas.

Experimental cultivars

'Hasansky Sladky' (*Vitis amurensis* × *V. labrusca* × *V. riparia* × *V. vinifera*) (synonyms: 'Varajane sinine', 'Baltica', 'Hasan Sweet') is Russian vigorous wine grape cultivar, yield ripens exceptionally early. Long small to medium sized slightly loose clusters (52 g) and small-medium blue-black berries (1.3 g) with low tannins. Quite disease resistant and it has good winter hardiness.

'Zilga' (*V. amurensis* × *V. labrusca* × *V. vinifera*) is Latvian early ripening wine and table grape cultivar. Small to medium semi-tight clusters (weight 138) and with medium (2.4 g) blue with sky-blue shade berries. Very vigorous and productive vine.

'Rondo' (*V. vinifera* × *V. amurensis*) is German wine and table grape cultivar with yield ripens medium. Medium sized blue berries (2.1 g) and clusters (118.6 g). Growth is vigorous. Susceptible for winter injuries.

Weather conditions

The average sum of active temperatures (≥10°C) for the years from 2009 to 2018 was 2304°C and ranged from 1981 to 2660°C (Table 1). The average length of the frost-free period was 158 days and ranged from 140 to 180 days. Half of the experimental years had the sum of active temperatures above the mean of 10 years. The warmest months were July and August when the average monthly sum of active temperatures was 578°C and 522°C respectively. The warmest spring was in 2013, 2016 and 2018. The highest sum of active temperatures in 2010, 2013, 2014, 2015 and 2018 was in August but in 2009, 2011, 2014 and 2018 in September. The last spring frost occurred usually at the beginning of May, except in 2009 and 2017 when it was in mid-May. The first autumn frost was mostly in the second half of October, but in four years, it was at the end of September or at the beginning of October. The 10 years average sum of precipitation from April to October was 462 mm. The amount of precipitation ranged from 325 to 566 mm. The phenological growth stages of grape identification scale (BBCH according to Lorenz et al. 1994) was used. The BBCH-scale for grapes describes the phenological development stages of grapes. The sums of active temperatures were calculated over the period of grape development before ripening (BBCH11-79) and at the time of ripening (BBCH81-89).

The Heliothermal Index (HI) was calculated using the following expression (Huglin 1978):

$$HI = \sum_{Mi}^{Mf} \left[\frac{(T - 10) + (Tmax - 10)}{2} \right] * d$$

where 'T' and 'Tmax' are, the average mean and maximum monthly temperature (°C), respectively; 'Mi' and 'Mf' are the initial and the final month of the period, respectively; 'd' is the length of day coefficient, with value of 1.09 for latitudes 58°.

Measurements and analysis

The juice soluble solids content (SSC; °Brix) from fresh berries was determined using a digital refractometer (Atago Pocket Refractometer Pal-1). Thirty grapes from each of the three replications from the different parts of clusters were analysed. A subsequent analyses were performed on previously frozen and then thawed berries. The titratable acids content (TAC) was determined in juice by the titration with 0.1 M NaOH solution to the endpoint of pH 8.2 (Wrolstad et al. 2005), using titrator Mettler Toledo EasyPlus Titration (with electrode DG 111-SC for endpoint detections). The TAC was expressed as g of tartaric acid per L of fresh juice. Four hundred grams of grapes from each of the three replications from the different parts of the cluster were analysed. The pH of grape juice was measured with a pH/conductivity-meter (HD 2156.1, Delta OHM). Tartaric (TA), malic (MA) and total acid (TOA) contents were estimated by FT-IR spectroscopy (Edelmann et al. 2003) and the measurements were carried out with FT-IR Wine & Must Analyzer

Table 1. The sum of active temperatures according to year, month and frost-free period, and the sum of precipitation from April to October in the years from 2009 to 2018.

	Sum of active temperatures (°C)									
Year	April	May	June	July	August	September	October	Total	Frost-free period (days)	Precipitation (mm)
2009	**75**	287	395	537	488	**374**	0	2156	147	**545**
2010	26	323	**448**	**700**	**572**	262	0	**2331**	148	**566**
2011	**72**	300	**531**	**636**	513	**376**	**71**	**2498**	**162**	325
2012	**51**	286	388	569	472	317	**99**	2181	**172**	**514**
2013	21	**435**	**547**	556	**533**	293	**105**	**2490**	150	352
2014	**98**	295	374	**605**	522	**332**	47	2274	140	**507**
2015	36	260	437	500	**529**	**348**	47	2156	149	398
2016	12	**427**	**461**	564	504	320	23	**2311**	**165**	**520**
2017	11	232	390	487	511	320	31	1981	156	**497**
2018	**72**	**443**	**456**	**630**	**573**	**365**	**121**	**2660**	**180**	399
Mean	47	329	443	578	522	331	54	2304	157	462

Note: Data according to the Estonian Environment Agency from Tartu-Tõravere weather station. Results in bold are higher than the mean of the 10 years.

(ALPHA, Bruker Optics, Ettlingen, Germany). The ratio between TA and MA was calculated by dividing.

Statistical analysis

The results of 'Hasansky Sladky', 'Rondo' and 'Zilga' were tested by one-way analysis of variance. To evaluate the effect of the year the least significant difference ($LSD_{0.05}$) was calculated and the different letters in tables mark a significant difference at $p \leq .05$. In 'Rondo' cultivation system experiment to evaluate the effect of treatment were tested by one-way analysis of variance. To evaluate the main effects of two factors (experimental year and treatment), the two-way analysis of variance was carried out, and marked as non-significant (ns) or using confidence levels as significant at $p \leq .05$*, .01** or .001***. Linear correlation coefficients were calculated between the variables ($n = 30$ 'Hasansky Sladky', $n = 26$ 'Rondo', $n = 27$ 'Zilga') with coefficient significance being $p \leq .05$*, and .01**. Relationship strength was estimated as $0.3 \leq r \leq 0.7$ (moderate), and $r \geq 0.7$ (strong).

Results

Heliothermal Index

HI ranged from 732 to 1422 in the years from 1987 to 2008, and in experimental years from 888 to 1532 (Figure 1). More than a half of the test years, the HI value was above the mean of 1222. In 2017, the HI was lowest and in 2018 the highest. The trend line shows warming, but HI had large variability between test years.

Soluble solids

The SSC was significantly different among years in a 10-year experiment – results ranged from 12.0 to 21.2 °Brix (Table 2). The SSC ranged from 17.1 to 21.2 °Brix in 'Hasansky Sladky' and the highest content was determined in 5 experimental years from 10. For 'Rondo', SSC ranged from 12.0 to 16.9 °Brix between the years. The lowest content was found in 2012 and 2017 (respectively 12.7 and 12.0 °Brix), but high content in four years. For 'Zilga', results ranged between 12.9 and 18.6 °Brix and the high SSC was in two years and low in three years from nine.

Vineyards cultivation systems had a significant positive effect on SSC in 'Rondo' (Table 3). In the tunnel conditions, SSC ranged from 15.2 to 17.8 °Brix, and in the open field from 12.0 to 16.6 °Brix. The SSC was significantly affected by the year ($p \leq .001$) and treatment ($p \leq .01$).

The SSC was higher in case of warmer weather conditions in April (mean) and May (mean and the sum of active temperatures) and with higher HI (Table 4). Growth periods BBCH11-79 and BBCH81-89 temperatures correlated positively with SSC in 'Rondo' and 'Zilga', but for 'Hasansky Sladky' only higher temperatures of BBCH11-79 period were important. Increase in precipitation decreased the SSC in grapes of 'Rondo' and 'Zilga'.

Acidity

The TAC had a large variation to do the impact of the year – ranged from 6.5 to 20.7 in 'Hasansky Sladky', from 9.2 to 22.7 in 'Rondo' and from 6.7 to 17.6 g L^{-1} in 'Zilga' (Table 2). For 'Hasansky Sladky' and 'Zilga', TAC was the lowest in 2018, for 'Rondo' also in 2016.

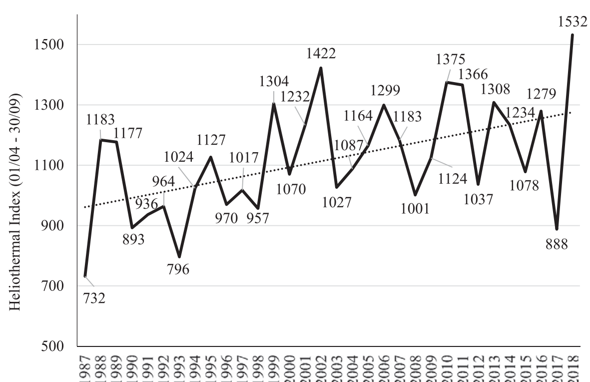


Figure 1. Heliothermal Index. Data according to AGRI4CAST (2019).

Table 2. The effect of growing year (2009–2018) on hybrid grape cultivars 'Hasansky Sladky', 'Rondo' and 'Zilga' technological maturity cultivated in open field conditions.

	'Hasansky Sladky'			'Rondo'			'Zilga'		
Year	SSC (°Brix)	TAC (g L^{-1})	pH	SSC °Brix	TAC (g L^{-1})	pH	SSC (°Brix)	TAC (g L^{-1})	pH
2009	21.0 a	14.3 d	3.2 e	14.3 b	14.8 b	2.9 d	16.0 cd	10.3 f	3.0 d
2010	20.7 a	16.1 b	3.2 e	13.6 bc	22.7 a	2.8 e	×	×	×
2011	18.5 bc	15.5 bc	3.2 e	16.8 a	14.1 bc	3.1 c	16.9 b	14.8 b	3.0 d
2012	17.1 c	20.7 a	2.9 g	12.7 cd	14.5 bc	3.1 c	13.5 e	13.7 c	2.9 e
2013	21.2 a	12.5 e	3.9 a	×	×	×	18.6 a	11.4 e	3.5 a
2014	20.4 a	12.6 e	3.5 c	×	×	×	15.6 d	12.4 d	3.3 b
2015	17.5 c	14.8 cd	3.3 d	16.9 a	13.8 c	3.6 a	15.3 d	17.6 a	3.5 a
2016	18.2 c	9.9 f	3.2 e	16.3 a	9.2 e	3.2 b	13.7 e	9.0 g	3.1 c
2017	17.2c	12.7 e	3.0 f	12.0 d	11.9 d	3.2 b	12.9 e	12.0 d	2.8 f
2018	20.7 a	6.5 g	3.6 b	16.6 a	11.9 d	3.2 b	18.0 a	6.7 h	3.1 c
$LSD_{0.05}$	1.7	0.8	0.1	1.5	0.8	0.1	1.3	0.6	0.1

Note: × – no results. SSC – soluble solids content, TAC – titratable acids content. Different letters in the same columns among years mark significant differences at $p \leq .05$.

Table 3. The effect of cultivation system on technological maturity and tartaric and malic acids of grape cultivar 'Rondo' (2016–2018).

Year	Cultivation system	SSC (°Brix)	TOA (g L^{-1})	pH	TA (g L^{-1})	MA (g L^{-1})	TA/MA
2016	Field	16.3 a	9.2 a	3.17 b	3.6 b	5.2 a	0.7 b
	Tunnel	17.0 a	7.9 b	3.35 a	4.3 a	2.9 b	1.5 a
	$LSD_{0.05}$	4.2	0.5	0.04	0.5	0.7	0.3
2017	Field	12.0 b	11.1 a	3.21 a	5.5 a	7.8 a	0.7 b
	Tunnel	15.2 a	8.3 b	2.95 b	4.1 b	3.6 b	1.2 a
	$LSD_{0.05}$	0.1	0.4	0.04	0.2	0.1	0.1
2018	Field	16.6 b	9.8 a	3.22 a	4.2 a	5.4 a	0.8 b
	Tunnel	17.8 a	6.5 b	3.27 a	3.7 a	1.9 b	2.0 a
	$LSD_{0.05}$	0.1	0.3	0.07	0.7	0.4	0.4
Year		***	***	***	***	***	**
Cultivation system		**	***	***	**	***	***

Note: SSC – soluble solids content, TOA – total acids content, TA – tartaric acid, MA – malic acid, TA/MA – tartaric to malic acid ratio. Different letters in the same columns among years according to the cultivation system mark significant differences of means at $p \leq .05$. The main effect of the year and cultivation system **, *** – significant at .01 or .001, respectively.

Table 4. The correlations between technological parameters and meteorological data for hybrid grape cultivars 'Hasansky Sladky', 'Rondo' and 'Zilga'.

	'Hasansky Sladky'			'Rondo'			'Zilga'		
	SSC (°Brix)	TAC (g L^{-1})	pH	SSC (°Brix)	TAC (g L^{-1})	pH	SSC (°Brix)	TAC (g L^{-1})	pH
April, SAT	0.311^{ns}	−0.002^{ns}	0.132^{ns}	0.315^{ns}	0.057^{ns}	−0.173^{ns}	0.377^{ns}	−0.038^{ns}	0.029^{ns}
May, SAT	0.442**	−0.627**	0.630**	0.474*	−0.280^{ns}	−0.088^{ns}	0.596**	−0.683**	0.286^{ns}
June, SAT	0.211^{ns}	−0.201^{ns}	0.495**	0.697**	−0.009^{ns}	0.025^{ns}	0.554**	−0.006^{ns}	0.340^{ns}
July, SAT	0.429*	−0.0002^{ns}	0.124^{ns}	0.163^{ns}	0.593**	−0.570**	0.553**	−0.326^{ns}	−0.030^{ns}
August, SAT	0.449**	−0.503**	0.535**	0.290^{ns}	0.383*	−0.029^{ns}	0.718**	−0.315^{ns}	0.391*
September, SAT	−0.081^{ns}	−0.225^{ns}	−0.045^{ns}	0.503**	−0.535**	0.299^{ns}	0.302^{ns}	−0.004^{ns}	−0.226^{ns}
April, mean	0.357*	−0.276^{ns}	0.309^{ns}	0.724**	0.082^{ns}	−0.008^{ns}	0.478*	−0.185^{ns}	0.359^{ns}
May, mean	0.509**	−0.661**	0.726**	0.395*	−0.254^{ns}	−0.055^{ns}	0.661**	−0.708**	0.332^{ns}
June, mean	0.196^{ns}	−0.414*	0.574**	0.739**	−0.342^{ns}	0.149^{ns}	0.561**	−0.166^{ns}	0.321^{ns}
July, mean	0.431*	−0.163^{ns}	0.202^{ns}	0.244^{ns}	0.482*	−0.475*	0.580**	−0.410*	−0.009^{ns}
August, mean	0.309^{ns}	−0.650**	0.528**	0.322^{ns}	0.129^{ns}	0.177^{ns}	0.545**	−0.345^{ns}	0.305^{ns}
September, mean	−0.105^{ns}	−0.566**	0.075^{ns}	0.539**	−0.682**	0.526**	0.276^{ns}	−0.355^{ns}	−0.169^{ns}
BBCH11-79	0.476**	−0.385*	0.549**	0.510**	0.135^{ns}	−0.295^{ns}	0.704**	−0.458*	0.270^{ns}
BBCH81-89	0.278^{ns}	−0.587**	0.376*	0.649**	−0.143^{ns}	0.226^{ns}	0.672**	−0.210^{ns}	0.108^{ns}
Year, SAT	0.477**	−0.456**	0.627**	0.605**	0.006^{ns}	−0.110^{ns}	0.834**	−0.417*	0.265^{ns}
HI	0.563**	−0.475**	0.550**	0.576**	0.169^{ns}	−0.259^{ns}	0.799**	−0.485*	0.268^{ns}
Frost-free period	−0.274^{ns}	−0.210^{ns}	−0.154^{ns}	0.165^{ns}	−0.486*	0.183^{ns}	0.154^{ns}	−0.411*	−0.482*
Precipitation	0.017^{ns}	0.213^{ns}	−0.523**	−0.639**	0.324^{ns}	−0.545**	−0.624**	−0.262^{ns}	−0.392*

Note: SSC – soluble solids content, TAC – titratable acids content; ns – non-significant correlation coefficient; significant at $p \leq .05$* or .01**; SAT – sum of active temperatures; mean – monthly mean temperature; BBCH11-79 – active temperatures of May, June, July; BBCH81-89 – active temperatures of August, September; HI – Heliothermal Index; frost-free period – sum of days where temperature was over 0°C; precipitation – monthly sum of precipitation (mm).

The highest content was found in 'Hasansky Sladky' in 2012, in 'Rondo' in 2010 and in 'Zilga' in 2015. The pH ranged between 2.8 and 3.9. Highest pH was in 'Hasansky Sladky' (3.9) in 2013, in 'Zilga' (3.5) in 2013 and 2015 and in 'Rondo' (3.6) in 2015. The lowest value was found in 2017 in cultivars 'Hasansky Sladky' and 'Zilga', but for 'Rondo' in 2010.

Growing conditions in tunnel decreased the TOA by 14%, 25.2% and 33.7% (respectively to the years) compared to open field (Table 3). In tunnel-grown grapes, the pH increased by 5.7% in 2016, but in 2017 decreased by 8.1%. TA ranged from 3.6 to 5.5 g L^{-1} and the impact direction of cultivation system differed; in 2016, tunnel cultivation increased the TA by 19.4%, but in 2017 decreased by 25.5% and in 2018 there was no effect. Growing grapes in tunnel reduced TA content by 2.3 in 2016, by 4.2 in 2017 and by 3.5 g L^{-1} in 2018. The vine cultivation system caused a large variation in TA and MA ratio (from 0.7 to 2.0) which was significantly higher in the tunnel in all years. All the tested technological maturity parameters were significantly affected by the year (TA/MA, $p \leq .01$; TOA, pH, TA and MA, $p \leq .001$) and cultivation system (TA, $p \leq .01$; TOA, pH, MA, and TA/MA, $p \leq .001$).

The TAC in 'Hasansky Sladky' and 'Zilga' was decreased with the higher sum of active temperatures of May ad year and with higher HI (Table 4). 'Rondo' had a moderate positive correlation with the mean and the sum of active temperatures in July, and the sum of active temperatures in August, but a moderately negative correlation with the mean and the sum of active temperatures in September. 'Rondo' and 'Zilga' needed a longer frost-free period to have a decrease in TAC. The pH in 'Hasansky Sladky' was increased in higher temperature conditions, but for other cultivars, higher temperatures had no significant effect. In all cultivars, pH decreased in higher precipitation conditions. However, all the cultivars had a moderate negative correlation with precipitation.

Discussion

In a 10-year experiment, high variability in SSC, TAC and pH values of hybrid grapevine fruits was found as shown in Table 2. With hybrid cultivars, the significant effect of yearly conditions has been found in other experiments as well (Lisek 2010; Gąstoł 2015). Suitable technological maturity for table grapes is 16 °Brix (OIV resolution Viti 1/2008) and for red table wines is provided when SSC ranges from 20.5 to 23.5 °Brix and pH is between 3.2 and 3.4 (Van Schalkwyk and Archer 2000). In this research, the obtained pH was at the desired level or near it in every year, but despite that a problem with sugar and acids content remained. It's in agreement with the literature that *labrusca* type hybrid grape cultivars like 'Hasansky Sladky' and 'Zilga' might not reach optimal maturity level for wine (Plocher and Parke 2008). These wine grapes can be harvested after SSC has reached °Brix from 17 to 18. Heymann et al. (2013) found that changes in fruit composition are more significant early in ripening for wine sensory attributes than after reaching 24 °Brix. Based on the experimental results 'Rondo' did not reach the desired sugar maturity level in any of the experimental years for winemaking, but for table grapes in four years. 'Hasansky Sladky' reached the desired wine level and above it in every year, but 'Zilga' only in three years. In five years 'Zilga' was suitable for table grapes. In Southern Finland, 'Zilga' has achieved sugar content 19 °Brix (Karvonen 2014) and 17.9 °Brix (Karvonen 2015). The mean SSC of experimental years in 'Rondo' grapes was 14.9 °Brix, but the mean of three years in Poland was 17.8 °Brix (Gąstoł 2015). SSC in 'Rondo' (16.3–16.8 °Brix) was highest in four years. Accumulation of soluble solids in 'Rondo' was temperature dependent – being enhanced by the mean and sum of active temperatures occurring in September, as higher temperature promoted it. 'Hasansky Sladky' and 'Zilga' had one of the highest SSC in 2013 and in 2018. In these years, the sum of active temperatures and HI were high, a frost-free period long and precipitation level low. In 2017, all the cultivars had one of the lowest SSC. The sum of active temperatures was lower than 10 years' mean and HI was below 1000.

In most of the experimental years, the TAC was higher than the desirable level for winemaking. Recommended titratable acids value is from 6.5 to 7.5 g L^{-1} (Van Schalkwyk and Archer 2000). Cultivars 'Hasansky Sladky' and 'Zilga' reached the desired level in 2018, but 'Rondo' none of the experimental years (Table 2). A decrease in the content of organic acids starts at the beginning of ripening and it is associated with changes in grape berry respiration (Volschenk et al. 2006). Higher temperatures enhance ripening and colourization of berries (due to the degradation of chlorophyll), which promotes the shift from sugar metabolism to malic acid respiration. Experimental cultivars reacted to temperature influences differently, because 'Hasansky Sladky' matures earlier when compared to 'Zilga' and 'Rondo'. Correlation analyses indicated that warm September for 'Rondo' and the longer frost-free period for 'Rondo' and 'Zilga' had a positive impact, but there was no effect on higher HI to 'Rondo' TAC, as presented in Table 4. This was caused by the peculiarity of the cultivar – 'Rondo' is late ripening cultivar and needs even higher HI. In a very cool climate, TAC is higher than in warmer climate conditions (Gladstones 1992). According to the literature, this is caused by high levels of MA (Conde et al. 2007), which is a problem in case of cool weather conditions in autumn. It is found that low night temperatures (10–11°C) around the *veraison* slows down the acid degradation, but low night temperatures after *veraison* have an irrelevant effect (Volschenk et al. 2006; Gaiotti et al. 2018). Wines made from grapes with higher organic acids content are sour and have more fresh vegetative flavours, and hybrid cultivars are recommended to be picked before the intensive *labrusca* flavour development (Plocher and Parke 2008).

Experimental results indicate that climate affects cultivars differently. For example 'Zilga' pH was less affected by different climatic parameters than 'Hasansky Sladky', the amount of precipitation affected SSC in 'Rondo' and 'Zilga'. In another study, the effect of year has been grape genotype dependent and affected by annual climatic conditions as well (Jackson and Lombard 1993). The experimental cultivars were different according to their properties such as winter resistance, cluster density and ripening time. For all cultivars, the warm spring was important by enhancing the beginning of vegetation period of the vine and promoting grape development. Consequently, the flowering time was also different and late spring frost could have affected it. For later ripening 'Rondo', the warmer temperatures in autumn were substantial. Temperatures in July affected the accumulation of acids due the optimum temperature range for acid synthesis is considered to be between 20°C and 25°C (Dokoozlian 2000). Cluster properties such as berry density could also have affected the impact of climate conditions – 'Rondo' and 'Zilga' berries are more tightly in the clusters than those of 'Hasansky Sladky'. The biochemical composition of grapes does not depend only on one but on a variety of environmental factors and their interaction (Conde et al. 2007; Dai et al. 2011). Favourable climatic conditions at pre-harvest are low precipitation, relatively high temperatures and long frost-free period, and according to these pre-mentioned conditions, 3 experimental years out of 10 were compatible for grape growing. Therefore, cultivars ripened at different times and the climate conditions during the grape maturation affected technological maturity accordingly.

Based on the HI classification by Tonietto and Carbonneau (2004), Estonia was according to calculations for every experimental year located in a very cool region. Except in 2018, when HI was for the first time in 10 years above 1500 and according to this classification Estonia could be classified to cool region. This indicates that due the climate conditions, the very early and early ripening cultivars could be suitable for growing in Estonia. The experiment showed that in Estonian very cool climate conditions, early ripening 'Hasansky Sladky' is suitable, but there is a problem with later ripening 'Rondo' and 'Zilga'. In high polyethylene tunnel cultivation system experiment, 'Rondo' showed an increase in SSC and decrease in TOA, bringing the contents to the desired level, as it can be observed from Table 3. In other experiments, the increase of soluble solids and decrease of total acids was caused by higher temperatures during ripening (Tarara et al. 2008; Mira de Orduña 2010). Higher temperatures in the tunnel probably have also affected the results of this experiment. The decrease of MA led to the normalised organic acid content.

The year had a significant impact on TA and MA (Table 3). For example in open field conditions, TA content was 35% higher in 2017 than in 2016. The change in the tunnel was 0.2 g L^{-1}. It is found that in a cool climate, the level of MA is usually higher when compared to warmer regions (Conde et al. 2007). In very cool climate conditions, MA could comprise up to 50% of the total acidity because the MA-related respiration is slower in a cooler temperature conditions (Volschenk et al. 2006). TA was less sensitive to the growing environment and in 2018 there was no effect of the tunnel. In general, TA concentration in grapes is more constant compared to MA (Volschenk et al. 2006). The higher MA is considered to be a problem in a cool climate and this was also confirmed in this experiment in very cool climate conditions. However, as demonstrated, growing vines in the tunnel can reduce the MA which decreased by 44–65% when compared to the open field conditions. In the process of respiration, the rate of MA metabolism depends on the temperature as higher temperatures at *veraison* and ripening stages reduce its content (Sweetman et al. 2014; Kizildeniz et al. 2018). In warmer weather conditions, grape cells increasingly use stored MA to satisfy their energy requirements for berry expansion.

In the open field conditions, the ratio of TA to MA in grape juice was from 0.7 to 0.8 and in the tunnel from 1.2 to 2.0, as presented in Table 3. Kliewer et al. (1967) defined four categories to evaluate the ratio of TA to MA: (1) high malate (below 1.20); (2) moderately high malate (1.21–1.75); (3) average malate (1.76–2.50); (4) low malate (above 2.51). According to this, open field grown grapes belong to high malate category and tunnel grapes moderately high malate to average malate category. Grapes harvested under different weather conditions and cultivation systems have diverse profile of organic acids and TA to MA ratio. In open field conditions, MA concentration was higher than TA, but in the tunnel, the concentration levels were opposite. It affected the TA to MA ratio. The ratio was more or almost twice as high in the tunnel-grown grapes as in the open field conditions. In wine cultivars, a high ratio of TA to MA improves the stability of wine (Liu et al. 2007). The ratio is higher in warmer climate, for example in Turkey, the mean ratio was found in *Vitis vinifera* cultivars to be 2.55 (Soyer et al. 2003), in the Czech Republic, the ratio differed between cultivars from 1.72 to 3.62 (Pavloušek and Kumšta 2011).

In conclusion, based on 10 years climate data assessment on macroclimate scale by Huglin's Heliothermal Index, experimental area is characterised by very cool climate class, which indicates the existence of the heliothermal potential for grapes maturation for very early and early cultivars. 'Hasansky Sladky' reached the desired level of SSC and above it every year in field experiment, but 'Zilga' only in three years out of nine. The SSC in 'Rondo' was near the lowest desired level in four years out of eight. Winemaking technology depends highly on the technological maturity of the berries. If the sugar concentration is low, it may be added and this addition is not subjected to local regulations in Estonia. In open field conditions, TAC in grapes of 'Hasansky Sladky' was higher than the recommended level in 8 years out of 10 in, 7 years out of 8 in 'Rondo' and 7 years out of 9 in 'Zilga'. TOA, TA and MA were at the appropriate level in tunnel-grown grapes of 'Rondo'.

Acknowledgements

The Estonian Environment Agency and AGRI4CAST are gratefully acknowledged for cooperation.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported by Estonian Science Foundation [grant number 9363], European Agricultural Fund for Rural Development (EAFRD) and Estonian Rural Development Plan (ERDP) for 2014–2020.

Notes on contributors

Mariana Maante-Kuljus is a researcher at the Chair of Horticulture in Estonian University of Life Sciences. She is a PhD student and her main research topics are viticulture and fruit biochemical composition. She has published articles on viticulture.

Reelika Rätsep (PhD in Agriculture) is a researcher at Polli Horticultural Research Centre of Estonian University of Life Sciences. Her PhD comprised fruit quality and cultivation (grapevine, strawberry). Main research focus in on fruit and berry quality and biochemical composition, especially antioxidants, and different extraction methods of bioactive. The second research interest is valorisation of plant-based raw materials, especially fruit and berry primary processing by-products.

Leila Mainla (Ph.D. in Agriculture) is an associate professor at the Chair of Horticulture in Estonian University of Life Sciences. Her PhD thesis focused on biochemical composition and storability of apples. Her field of research currently is horticulture focusing on ornamentals (growing technology of annuals, perennials and woody ornamentals). Currently leading two projects on that subject.

Ulvi Moor, PhD is an associate professor at the Chair of Horticulture, Estonian University of Life Sciences. Her current research interests are out of season berry production in Nordic countries and preharvest factors affecting postharvest performance of horticultural crops.

Marge Starast is an associate professor of Plant physiology at the Chair of Horticulture, Estonian University of Life Science, where she teaches plant physiology of horticultural crops and biotechnology. She received a PhD degree in botany and mycology from Tartu University, Estonia. Her scientific work focuses on the plant cultivation technologies and these impact on the chemical composition of plant. She has published more than 30 scientific articles in English. She is also referee/reviewer of seven international scientific journal.

Priit Põldma is a lecturer at the Chair of Horticulture, Estonian University of Life Sciences. His main research interests are cultivation technologies, quality and postharvest life of horticultural crops. Currently he is leading four national research projects and is participating in one international research project.

Kadri Karp is Professor of Horticulture, PhD at the Chair of Horticulture, Estonian University of Life Sciences, Estonia. Kadri Karp has supervised seven doctor's dissertations, is the author of over 30 scientific articles, coordinator of three research projects (in progress); her field of research is agricultural sciences and speciality is horticulture. The research topics are focusing on antioxidant compounds, horticultural technologies and wine grapes (maturity parameters, viticultural technologies and wine).

ORCID

Reelika Rätsep http://orcid.org/0000-0002-8673-9978
Ulvi Moor http://orcid.org/0000-0002-8808-8494
Priit Põldma http://orcid.org/0000-0002-7906-823X

References

AGRI4CAST. 2019. Gridded agro-meteorological data in Europe. [accessed 2019 Feb 10]. http://agri4cast.jrc.ec.europa.eu/.

Baiano A, De Gianni A, Previtali MA, Del Nobile MA, Novello V, De Palma L. 2015. Effect of defoliation on quality attributes of Nero di Troia (*Vitis vinifera* L.) grape and wine. Food Res Int. 75:260–269. doi:10.1016/j.foodres.2015.06.007.

Conde C, Silva P, Fontes N, Dias ACP, Tavares RM, Sousa MJ, Agasse A, Delrot S, Gerós H. 2007. Biochemical changes throughout grape berry development and fruit and wine quality. Food. 1(1):1–22.

Dai ZW, Ollat N, Gomès E, Decroocq S, Tandonnet J-P, Bordenave L, Pieri P, Hilbert G, Kappel C, van Leeuwen C, et al. 2011. Ecophysiological, genetic, and molecular causes of variation in grape berry weight and composition: a review. Am J Enol Vitic. 62(4):413–425. doi:10.5344/ajev.2011.10116.

Dokoozlian NK. 2000. Grape berry growth and development. In: Rasin production manual. Oakland: University of California. Agricultural and Natural Resources Publication; p. 30–37.

Edelmann A, Diewok J, Baena JR, Lendl B. 2003. High-performance liquid chromatography with diamond ATR-FTIR detection for the determination of carbohydrates, alcohols and organic acids in red wine. Anal Bioanal Chem. 376(1):92–97. doi:10.1007/s00216-003-1879-0.

Gaiotti F, Pastore C, Filippetti I, Lovat L, Belfiore N, Tomasi D. 2018. Low night temperature at veraison enhances the accumulation of anthocyanins in Corvina grapes (*Vitis vinifera* L.). Sci Rep. 8:8719. doi:10.1038/s41598-018-26921-4.

Gąstoł M. 2015. Vineyard performance and fruit quality of some interspecific grapevine cultivars in cool climate conditions. Folia Hortic. 27:21–31. doi:10.1515/fhort-2015-0011.

Gladstones J. 1992. Viticulture and environment. Adelaide, SA: Winetitles.

Gustafsson J-G, Mårtensson A. 2005. Potential for extending Scandinavian wine cultivation. Acta Agric Scand B Soil Plant Sci. 55:82–97. doi:10.1080/09064710510029097.

Heymann H, LiCalzi M, Conversano MR, Bauer A, Skogerson K, Matthews M. 2013. Effects of extended grape ripening with or without must and wine alcohol manipulations on Cabernet Sauvignon wine sensory characteristics. S Afr J Enol Vitic. 34(1):86–99. doi:10.21548/34-1-1084.

Huglin P. 1978. New way to evaluate the solar thermal possibilities of a wine growing environment. Proc French Acad Agric. 64:1117–1126. French.

Hunter JJ, Volschenk CG, Marais J, Fouché GW. 2004. Composition of Sauvignon Blanc grapes as affected by prevéraison canopy manipulation and ripeness level. S Afr J EnolViticult. 25(1):13–18. doi:10.21548/25-1-2132.

Jackson D, Lombard PB. 1993. Environmental and management practices affecting grape composition and wine quality: a review. Am J Enol Vitic. 44:409–430.

Jones GV, Duff AA, Hall A, Myers J. 2010. Spatial analysis of climate in winegrape growing regions in the western United States. Am J Enol Vitic. 61:313–326.

Karvonen J. 2014. *Vitis* cv. Zilga is a vine for the northern temperate climate – short communication. Hortic Sci. 41:147–151. doi:10.17221/17/2014-HORTSCI.

Karvonen J. 2015. Phenolic compounds of grape varieties grown in the northern temperate climate. Int J Agric Innov Res. 4(2):311–316.

Karvonen J. 2017. Impact of climate change on winegrowing conditions in southernmost Finland (Tuusula). Int J Enol Viticult. 4(4):169–178.

Kizildeniz T, Pascual I, Irigoyen JJ, Morales F. 2018. Using fruit-bearing cuttings of grapevine and temperature gradient greenhouses to evaluate effects of climate change (elevated CO_2 and temperature, and water deficit) on the cv. red and white Tempranillo. Yield and must quality in three consecutive growing seasons (2013–2015). Agric Water Manag. 202:299–310. doi:10.1016/j.agwat.2017.12.001.

Kliewer WM. 1966. Sugars and organic acids of *Vitis vinifera*. Plant Physiol. 41:923–931.

Kliewer WM. 1971. Effect of day temperature and light density on concentration of malic and tartaric acids in *Vitis vinifera* L. grapes. Am J Enol Vitic. 96:372–377.

Kliewer WM, Howarth L, Omori M. 1967. Concentrations of tartaric acid and malic acids and their salts in *Vitis vinifera* grapes. Am J Enol Vitic. 18:42–54.

Lisek J. 2010. Yielding and healthiness of selected grape cultivars for processing in central Poland. J Fruit Ornam Plant Res. 18(2):265–272.

Liu HF, Wu BH, Fan PG, Xu HY, Li SH. 2007. Inheritance of sugars and acids in berries of grape (*Vitis vinifera* L.). Euphytica. 153:99–107. doi:10.1007/s10681-006-9246-9.

Lorenz DH, Eichhorn KW, Bleiholder H, Klose R, Meier U, Weber E. 1994. Phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. *vinifera*)—codes and descriptions according to the extended BBCH. Die Weinwissenschaft Vitic Enol Sci. 49:66–70. German.

Maante M, Vool E, Karp K. 2016. Effect of defoliation on grape maturity parameters. Sodininkyste ir Darzininkyste. 35:21–35.

Mira de Orduña R. 2010. Climate change associated effects on grape and wine quality and production. Food Res Int. 43:1844–1855. doi:10.1016/j.foodres.2010.05.001.

OIV resolution Viti 1/. 2008. OIV standard on minimum maturity requirements for table grapes. www.oiv.int/en/technical-standards-and-documents/resolutions-of-the-oiv/viticulture-resolutions.

Pavloušek P, Kumšta M. 2011. Profiling of primary metabolites in grapes of interspecific grapevine varieties: sugars and organic acids. Czech J Food Sci. 29(4):361–372. doi:10.17221/257/2010-CJFS.

Plocher T, Parke B. 2008. Northern wineworks: growing grapes and making wine in cold climates. 2th ed. Hugo, MN: Northern Winework.

Rätsep R, Karp K, Vool E, Tõnutare T. 2014. Effect of pruning time and method on hybrid grapevine (Vitis sp.) 'Hasanski Sladki' berry maturity in a cool climate conditions. Acta Sci Pol Hortoru Cultus. 13:99–112.

Ruffner HP. 1982. Metabolism of tartaric and malic acids in *Vitis*: a review – part A. Vitis. 21:247–259.

Soyer Y, Koca N, Karadeniz F. 2003. Organic acid profile of Turkish white grapes and grape juices. J Food Compos Anal. 16:629–636. doi:10.1016/S0889-1575(03)00065-6.

Sweetman C, Deluc LG, Cramer GR, Ford CM, Soole KL. 2009. Regulation of malate metabolism in grape berry and other developing fruits. Phytochemistry. 70(11–12):1329–1344. doi:10.1016/j.phytochem.2009.08.006.

Sweetman C, Sadras VO, Hancock RD, Soole KL, For CM. 2014. Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. J Exp Bot. 65 (20):5975–5988. doi:10.1093/jxb/eru343.

Tarara MJ, Lee J, Spayd ES, Scagel FC. 2008. Berry temperature and solar radiation alter acylation, proportion, and concentration of anthocyanin in Merlot grapes. Am J Enol Vitic. 59:235–247.

Tonietto J, Carbonneau A. 2004. A multicriteria climatic classification system for grape-growing regions worldwide. Agric For Meteorol. 124:81–97. doi:10.1016/j.agrformet.2003.06.001.

Van Schalkwyk H, Archer E. 2000. Determining optimum ripeness in wine grapes. Wineland. 130:90–91.

Volschenk H, van Vuuren HJJ, Viljoen-Bloom M. 2006. Malic acid in wine: origin, function and metabolism during vinification. S Afr J Enol Viticult. 27(2):123–136. doi:10.21548/27-2-1613.

Wrolstad RE, Acree TE, Decker EA, Penner MH, Reid DS, Schwartz SJ, Shoemaker CF, Denise MS, Sporns P, editors. 2005. Handbook of food analytical chemistry. Pigments, colorants, flavors, texture, and bioactive food components. New Jersey: Wiley. 624 p.

IV

Maante-Kuljus, M., Rätsep, R., Moor, U., Mainla, L., Põldma, P., Koort, A., Karp, K. 2020. Effect of vintage and viticultural practices on the phenolic content of hybrid winegrapes in very cool climate. *Agriculture,* 10(5): 169. doi:10.3390/agriculture10050169 agriculture

MDPI

Article

Effect of Vintage and Viticultural Practices on the Phenolic Content of Hybrid Winegrapes in Very Cool Climate

Mariana Maante-Kuljus [1,*], Reelika Rätsep [2,3], Ulvi Moor [1], Leila Mainla [1], Priit Põldma [1], Angela Koort [1] and Kadri Karp [1]

[1] Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1, 51006 Tartu, Estonia; ulvi.moor@emu.ee (U.M.); leila.mainla@emu.ee (L.M.); priit.poldma@emu.ee (P.P.); angela.koort@emu.ee (A.K.); kadri.karp@emu.ee (K.K.)
[2] Institute of Agricultural and Environmental Sciences, Polli Horticultural Research Centre, 69108 Polli, Estonia; reelika.ratsep@emu.ee
[3] Institute of Veterinary Medicine and Animal Sciences, ERA-Chair Valortech, Kreutzwaldi 56/5, 51014 Tartu, Estonia
* Correspondence: mariana.maante-kuljus@emu.ee

Received: 17 April 2020; Accepted: 11 May 2020; Published: 14 May 2020

check for updates

Abstract: Vine growing and wine production is gaining in popularity around the Baltic Sea Region. The first aim of the experiment was to determine the variability of the total phenolic and anthocyanin content (from 2010 to 2018) and of individual anthocyanin content (from 2016 to 2018) in the hybrid grape cultivars 'Hasansky Sladky', 'Zilga', and 'Rondo'. In field conditions 'Rondo' had winter cold damage to canes in two years. Therefore, the second aim was to determine the effect of high polyethylene tunnel and field conditions on fruit total and individual anthocyanin content of 'Rondo' from 2016 to 2018. Over nine years, the total phenolic content ranged from 192 to 671 mg 100 g^{-1} and anthocyanins from 30 to 405 mg 100 g^{-1} spectrophotometrically. The anthocyanin (delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside, malvidin-3-O-glucoside) content depended on cultivar properties and climatic parameters. Antioxidant activity was cultivar dependent and ranged from 40 to 88%. Polytunnel cultivation increased the content of total anthocyanins in 'Rondo' from 447 to 1472 mg 100 g^{-1} (by chromatographically) in cooler year, but in warmer years it decreased from 3645 to 1618 mg 100 g^{-1}. Individual anthocyanins showed the same tendency. Grapevine cultivar 'Rondo' is recommended for tunnel growing in very cool climate conditions.

Keywords: anthocyanins; antioxidant activity; cyanidin; delphinidin; malvidin; peonidin; petunidin; *Vitis* hybrids

1. Introduction

Polyphenols are important for wine color intensity, astringency, and bitterness. The phenolic content and profile of grapes depends on cultivar, growing area, climatic conditions, and viticultural practices [1–7]. Cultivars with a higher total phenolic content tend to have higher antioxidant activity [8,9]. Anthocyanins are the main compounds responsible for the red color of grapes and they are synthesized to protect the skin from the negative effect of the environment, especially ultraviolet radiation. The anthocyanin composition depends on the genetic background of *Vitis* species [10]. However, it has also been found to be affected by cultivar, climate [11], and different viticultural practices [12,13]. Elevated temperatures during ripening may reduce the accumulation of anthocyanins and could partly degrade the previously synthesized components [14,15]. Therefore, anthocyanin accumulation in hot regions is inhibited in the skins of red and black grapes, but further north conditions are more favorable. In wines produced from hybrid cultivars, malvidin was the most abundant compound, accounting for 58 to 62% of the pigments [16]. The anthocyanin profile of wine has been used as a tool to assess the varietal origin of single cultivar wines, being called the "anthocyanin fingerprint" [17]. Hybrids differ in their phenolic and anthocyanin profiles from *Vitis vinifera* cultivars [2–4]. So far the phenolic compounds of cultivars 'Hasansky Sladky' and 'Zilga' have been found to be affected by viticultural practices such as defoliation [18] and pruning methods [19]. There are no long-term studies on how very cool climatic conditions affect the phenolic composition of cultivars 'Hasansky Sladky', 'Zilga', and 'Rondo'.

Hybrid grape cultivars are suitable for growing in cool climate conditions [20]. In Finland, 'Zilga' and 'Rondo' have been tested in the field [21,22] and have potential as high quality wine grapes. In Estonia, vine growing has intensified in recent years and winemaking even more. According to the Heliothermal Index, Estonia belongs to a very cool vine growing area [23]. In this region, 'Hasansky Sladky', and 'Zilga' in the field reached desired soluble solids content, but 'Rondo' did not.

The first aim of this study was to determine the variability of the total phenolic and anthocyanin content over a period of 2010–2018 and of individual anthocyanin content over the years 2016−2018 in the hybrid grape cultivars 'Hasansky Sladky', 'Zilga', and 'Rondo'. The second aim was to determine the effect of high polyethylene tunnel and field conditions on fruit total and individual anthocyanins over the years 2016−2018 in 'Rondo'.

2. Materials and Methods

2.1. Experimental Sites and Plant Material

'Hasansky Sladky', 'Zilga', and 'Rondo' were investigated in field conditions in a period of 2010–2018. The effect of polytunnel viticultural practice in 'Rondo' was investigated in 2016–2018 period. The polytunnel (58°17′1″ N, 26°33′41″ E) was 28 m long, 7.6 m wide, and 4.6 m high, covered with 0.18 mm thick UV stable low-density polyethylene (direct photosynthetically active radiation 88–90) and was situated 8.5 km from the field vineyard (58°17′1″ N, 26°33′41″ E). At both sites, the vine rows were oriented from north to south. Vines of tunnel were planted in spaces of 1.6 × 2 m and in field 2 × 2 m. Both vineyards were established in 2007 with own-rooted plants. The experimental design was randomized block with 4 replicates and 8 vines in each. In 2010, field grown 'Zilga' had spring frost damage to flowers, and 'Rondo' had winter cold damage to canes in 2013 and 2014. In table "×" marks years without harvest in these cultivars.

The soil of both experimental areas was sandy loam *Haplic Luvisol*. Soils were sufficiently drained and soil fertility was 45 to 50 points in 100-point scale. The soil nutrient content in the field was: P and Mg—excessive, K—high, Ca—medium and pH_{KCl} was 5.4 (Table 1). P, K, Ca, and Mg values in the tunnel were high and pH_{KCl} was 5.4.

Table 1. Nutrient content (mg kg^{-1}) of the soil in two experimental areas.

Viticultural Practice	P	K	Mg	Ca	pH_{KCl}
Field	147	257	260	1670	5.4
Tunnel	159	578	574	2381	5.4

Experimental cultivars:

'Hasansky Sladky' (*Vitis amurensis* Ruprecht × 'Dalnevostochnyi Tikhonova') [24] (synonyms: 'Hasan Sweet', 'Varajane Sinine', 'Baltica') is a vigorous Russian winegrape cultivar witch ripens exceptionally early. It has long, small to medium-sized slightly loose clusters and small-medium blue-black berries. It is quite disease resistant and has good winter hardiness.

'Zilga' [('Smuglyanka' × 'Dvietes') × 'Jublinaja Novgoroda')] [25] is a Latvian early ripening wine and table grape cultivar. It has small to medium semi-tight clusters and medium blue to sky-blue shade berries. It is a very vigorous and productive vine.

'Rondo' ('Zarya Severa' × 'Saint Laurent') [26] is a German wine and table grape cultivar with medium ripening yield. It has medium sized blue berries and clusters. Plant growth is vigorous.

2.2. Vineyard Management

For establishment of the experiments at both sites the ground was covered with black synthetic mulch, and no irrigation system was established. No additional fertilizers were used at either experimental area. White polypropylene fabric was used in tunnel as winter cover. Vines were trained on low double trunk trellis with 12 buds left per plant. Branches were pruned after sap flow was ended in spring. After formation of inflorescence, shoots were thinned (fruitless shoots were removed). Once every two weeks, lateral shoots and main shoots were cut back. Shoots height was eight leaves after clusters. About four leaves from the cluster zone were removed at the beginning of *veraison*. After *veraison* soluble solids content was determined once a week on randomly chosen grapes from basal clusters. The harvest time was determined when soluble solids content did not change significantly or due to the weather conditions (arrival of night frosts). Depending on year, the technological maturity parameters at harvest in field conditions were: 'Hasansky Sladky'—soluble solids content (SSC) 17 to 21 °Brix, titratable acids content (TAC) 6.5 to 20.7 G tartaric acid L^{-1}, pH 2.9 to 3.6; 'Rondo' SSC 12 to 17 °Brix, TAC 9.2 to 22.7 G tartaric acid L^{-1}, pH 2.8 to 3.6; 'Zilga' SSC 13 to 19 °Brix, TAC 6.7 to 17.6 G tartaric acid L^{-1}, pH 2.8 to 3.5 [23]. In tunnel conditions: 'Rondo' SSC was 15 to 18 °Brix, TAC 6.5 to 8.3 G tartaric acid L^{-1}, pH 3 to 3.4. The average weight of 'Hasansky Sladky' berry was 1.3 g and cluster 52 g, 'Rondo' respectively 2.1 g and 119 g, and 'Zilga' respectively 2.4 g and 138 g.

2.3. Weather Conditions

Weather data was obtained from The Estonian Environment Agency Tartu-Tõravere meteorological station (located 11 km from the field and 6 km from tunnel). The air temperatures, precipitation, and relative air humidity (RH, %) were recorded in the observation area 24 h a day, every hour. The tunnel air temperature was recorded with temperature data loggers 24 h a day, every hour. In most experimental years, the warmest month was July, except in 2015 and 2017 when August was warmer (Table 2). In spring months the mean temperature in May was 12.5 °C and in June 15.2 °C. At the time of *veraison* mean temperature in July was 18.5 °C, in August 16.7 °C and in September 12.2 °C. Mean monthly temperatures in 2018 were higher in every month compared to the mean of experimental years. Temperatures in tunnel conditions were higher—depending on month 0.6 to 5.9 °C. There was a drought in May 2016 and 2018. Autumn months in 2016 and 2017 differed from other years by the severity of precipitation. Additionally, the air humidity was higher in these years.

The sum of active temperatures (SAT) was calculated by summing the daily average temperatures above 10 °C (monthly, year). The grape phenological growth stage identification scale (BBCH) was used in phenological observations [27]. Phenological observations were made once a week over the vegetation period (April to October) on the basal cluster of each plant. Over the period of grape development (SAT BBCH 71–79, June–July) and at the time of *veraison* (SAT BBCH 81–89, August–September) SAT was calculated. The radiation flux (RF, W m^2) data was obtained from the Tartu University Laboratory of Environmental Physics (10 km from field and 12 km from tunnel). RF was recorded in the observation area 24 h a day, every five minutes. The monthly average was calculated. RF for the years from 2016 to 2018 was 153 to 183 W m^2 in August and in September 93 to 120 W m^2.

Table 2. Monthly temperatures, precipitation and relative air humidity from April to October in the years from 2010 to 2018 in field conditions and the monthly temperatures in tunnel from 2016 to 2018.

	Year	Mean Temperature (°C)						
		April	**May**	**June**	**July**	**August**	**September**	**October**
Field	2010	**5.7**	12.2	14.3	**21.7**	**17.8**	10.7	3.8
	2011	**5.7**	11.0	**17.2**	**19.9**	15.8	**12.3**	**6.8**
	2012	4.6	11.4	13.3	17.7	14.7	11.9	5.3
	2013	4.0	**15.5**	**17.8**	17.5	16.6	10.8	**6.6**
	2014	**6.8**	12.3	13.7	**19.5**	**16.8**	**12.5**	**5.6**
	2015	**5.8**	10.6	14.6	16.1	**17.0**	**12.8**	4.9
	2016	**5.9**	**14.1**	**16.3**	18.2	16.3	**12.5**	4.2
	2017	1.6	10.2	13.8	15.7	16.5	12.1	5.2
	2018	**7.2**	**15.2**	**15.5**	**20.2**	**18.5**	**14.0**	**7.2**
	Mean	5.3	12.5	15.2	18.5	16.7	12.2	5.5
Tunnel	2016	6.9	18.0	**20.5**	22.0	18.6	13.4	5.2
	2017	4.6	16.1	17.1	20.6	18.8	12.7	6.0
	2018	**10.2**	**20.1**	**20.4**	**23.6**	**20.8**	**15.6**	**8.6**
	Mean	7.2	18.1	19.3	22.1	19.4	13.9	6.6
		Precipitation (mm)						
Field	2010	25	**97**	**98**	38	**148**	**99**	**59**
	2011	1	**58**	35	48	55	**80**	48
	2012	**45**	**78**	**98**	**80**	80	61	**72**
	2013	36	**65**	29	**67**	73	38	45
	2014	16	**90**	**134**	**78**	**126**	20	43
	2015	**80**	**61**	66	**68**	47	**67**	8
	2016	**70**	2	**207**	**86**	**104**	15	37
	2017	29	28	65	57	**112**	**119**	**86**
	2018	**43**	10	66	23	81	**99**	**78**
	Mean	38	54	89	61	92	64	53
		Relative air humidity (%)						
	2010	68	**72**	**72**	68	**79**	**84**	83
	2011	65	**66**	65	72	75	81	81
	2012	65	61	69	73	**81**	**84**	**91**
	2013	**69**	**69**	**71**	72	73	80	84
	2014	53	**69**	**76**	70	76	77	78
	2015	**75**	**71**	70	**77**	75	**86**	84
	2016	**73**	61	**71**	**80**	**83**	**84**	84
	2017	**75**	62	**71**	**76**	77	**85**	**87**
	2018	67	54	63	73	74	81	**88**
	Mean	68	65	70	73	77	82	84

Note: Data according to the Estonian Environment Agency from Tartu-Tõravere weather station. Tunnel temperature data were collected from temperature data logger. Results in bold are higher than the mean of all experimental years.

The Heliothermal Index (HI) was calculated using the following expression (Huglin [28]):

$$HI = \sum_{Mi}^{Mf}\left[\frac{(T-10)+(T_{max}-10)}{2}\right]\times d \quad (1)$$

where ‘T’ and ‘T_{max}’ are, the average mean and maximum monthly temperature (°C), respectively; ‘Mi’ and ‘Mf’ are the initial and the final month of the period, respectively; ‘d’ is the length of day coefficient, with value of 1.09 for latitudes 58°.

2.4. Measuerments and Analysis

Samples of grapes were collected from all experimental cultivars at harvest from the field in 2010 to 2018 and from ‘Rondo’ in the tunnel in 2016 to 2018. For phenolic compounds samples (three replications) of 400 g from the different parts of the basal cluster for analyses were collected from every cultivar/viticultural practice. From one replication three separate extractions were made from grape skins (exocarp of fruit). From 2010 to 2018, at harvest, samples were collected from the field.

The total phenolic content (TPC) was determined by applying the Folin-Ciocalteau phenol reagent method [29]. Ethanol-acetone (7:3) solution was used as the solvent to extract the total phenolic compounds (5 g of berries skins was added 50 mL of solution). TPC was expressed as mg of gallic acid equivalent per 100 g of fresh skins weight (FSW). Total anthocyanin content (ACC_{spec}) was determined with the pH-differential method [29]. Hydrochloric acid-ethanol (15:85) solution was used as the solvent to extract the ACC_{spec} (10 g of berry skins was added 100 mL of solution). ACC_{spec} was expressed as mg of cyanidin-3-glucoside equivalent per 100 g of FSW. In 2016 to 2018 samples, the total antioxidant activity (TAA) was determined by applying the DPPH (2.2-diphenyl-1-picrylhydrazyl) radical scavenging method [30]. TAA was expressed as %. Spectrophotometric measures were made with UVmini-1240 Shimadzu (Shimadzu, Kyoto, Japan).

Total anthocyanin (ACC_{HPLC}) and individual anthocyanins (Figure 1) were determined using the method for polyphenol profiling [31]. Samples were prepared in three replicates; approx. 1 g of berry skin sample was added to 50% ethanol + 1% HCl (v:v) solution. Chromatographic analyses (HPLC) were made with Shimadzu Nexera X2 (Shimadzu, Kyoto, Japan) at a wavelength of 520 nm. The results of total ACC_{HPLC} were expressed as malvidin-3-O-glycoside equivalent mg 100 g^{-1} of FSW. The major anthocyanins were delphinidin-3-O-glucoside (Dp), cyanidin-3-O-glucoside (Cy), petunidin-3-O-glucoside (Pt), peonidin-3-O-glucoside (Pn), and malvidin-3-O-glucoside (Mv).

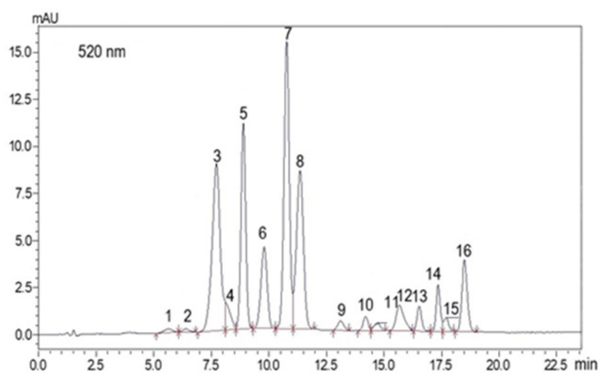


Figure 1. An example of anthocyanins profile of 'Rondo': 1—cyanidin-3-O-glucoside-5-O-glucoside; 2—petunidin-3-O-glucoside-5-O-glucoside; 3—delphinidin-3-O-glucoside; 4—malvidin-3-O-glucoside-5-O-glucoside; 5—cyanidin-3-O-glucoside; 6—petunidin-3-O-glucoside; 7—peonidin-3-O-glucoside; 8—malvidin-3-O-glucoside; 9—delphinidin-3-O-(6″-O-acetyl)-glucoside; 10—cyanidin-3-O-(6-O-acetyl)-glucoside; 11—petunidin-3-O-(6″-O-acetyl)-glucoside; 12—malvidin-3-O-(6-O-acetyl)-glucoside; 13—delphinidin-3-O-(6″-p-coumaroyl)-glucoside; 14—cyanidin-3-O-(6″-p-coumaroyl)-glucoside; 15—petunidin-3-O-(6″-O-coumaroyl)-glucoside; 16—peonidin-3-O-(6″-O-coumaroyl)-glucoside.

2.5. Statistical Analysis

The results of measured parameters for 'Hasansky Sladky', 'Rondo' and 'Zilga' were tested by one-way analysis of variance. The least significant difference ($LSD_{0.05}$) was calculated to evaluate the effect of year and different letters in tables mark significant differences at $p \leq 0.05$. The effect of cultivar was tested by one-way analysis of variance. To evaluate the main effect of two factors (experimental, year and cultivar and the interaction between them), the two-way analyses of variance was carried out, and results marked as non-significant (ns) or using confidence level significance at $p \leq 0.05$ *, $p \leq 0.01$ ** and $p \leq 0.001$ ***. The effect of treatment in the 'Rondo' viticultural practice experiment was tested by one-way analysis of variance. To evaluate the main effect of two factors (year and viticultural practice and the interaction between them), the two-way analyses of variance was carried out, and results marked as non-significant (ns), or using confidence level significant at $p \leq 0.05$ *, $p \leq 0.01$ ** and $p \leq 0.001$ ***. Linear correlation coefficients were calculated between the variables (n = 9 'Hasansky Sladky', 'Rondo', 'Zilga') with coefficient significance being $p \leq 0.05$ *, and 0.01 **. Relationship strength was estimated $0.3 \leq r \leq 0.7$ moderate, and $r \geq 0.7$ strong. Principal component analysis (PCA) was applied to the chemical composition of grapes and meteorological data to study the possible grouping of the cultivars and year.

3. Results

3.1. Climate Conditions

The average SAT for the BBCH 71–79 was 1031 °C and ranged from 877 to 1167 °C (Table 3). For the SAT BBCH 81–89 the average was 851 °C, ranging from 789 to 938 °C. The average SAT for the years from 2010 to 2018 was 2320 °C and ranged from 1981 to 2660 °C. Four years out of nine the SAT exceeded the mean value of all years. HI had large variability in test years and ranged from 888 to 1532. Estonia belongs to in a very cool vine growing region, except in 2018, when HI was above 1500 and according to this Estonia could be classified to cool region. The average length of the frost-free period was 158 days, ranging from 140 to 180 days. The last spring frost usually occurred at the beginning of May, except in 2017 when it was in mid-May. The first autumn frost occurred mostly in the second half of October, but in four years, it was at the end of September or beginning of October. The amount of precipitation ranged from 325 to 566 mm and the rainiest years was 2010.

Table 3. The sum of active temperatures, Heliothermal Index, frost-free period, and the precipitation (April–October, 2010–2018).

	SAT (°C)					
Year	**BBCH 71–79**	**BBCH 81–89**	**Total**	**HI**	**Frost-Free Period (Days)**	**Precipitation (mm)**
2010	**1148**	834	**2331**	**1375**	148	**566**
2011	**1167**	**889**	**2498**	**1366**	**162**	325
2012	957	789	2181	1037	**172**	**514**
2013	**1103**	826	**2490**	**1308**	150	352
2014	979	**854**	2274	**1234**	140	**507**
2015	937	**877**	2156	1078	149	398
2016	1025	824	2311	**1279**	**165**	**520**
2017	877	831	1981	888	156	**497**
2018	**1086**	**938**	**2660**	**1532**	**180**	399
Mean	1031	851	2320	1233	158	453

Note: SAT—sum of active temperatures (≥10 °C); BBCH − phenological growth stage identification scale; BBCH 71–79—active temperatures of June, July; BBCH 81–89—active temperatures of August, September; HI—Heliothermal Index. Data according to the Estonian Environment Agency from Tartu-Tõravere weather station. Results in bold are higher than the mean of experimental years.

3.2. Phenolic Compounds

The TPC had a large variation due to the effect of year—TPC in 'Hasansky Sladky' ranged from 192 to 394 mg 100 g^{-1}, in 'Rondo' from 374 to 671 mg 100 g^{-1} and in 'Zilga' from 214 to 372 mg 100 g^{-1} (Table 4). Each cultivar had its highest content in a different year—'Hasansky Sladky' in 2011, 'Rondo' in 2018, and 'Zilga' in 2014 and 2016. The TPC was significantly affected by year, cultivar, and interaction between them ($p \leq 0.001$).

Table 4. The effect of year and cultivar on phenolic compounds of grape cultivars 'Hasansky Sladky', 'Rondo' and 'Zilga' (2010–2018) cultivated in field conditions.

Year	'Hasansky Sladky'		'Rondo'		'Zilga'	
	TPC	**ACC_{spec}**	**TPC**	**ACC_{spec}**	**TPC**	**ACC_{spec}**
	mg 100 g^{-1} FSW					
2010	279 d	133 a	399 cd	112 d	×	×
2011	394 a	74 c	482 b	134 bc	293 b	54 e
2012	192 h	113 d	374 d	75 e	214 e	50 e
2013	326 b	138 a	×	×	222 de	64 d
2014	253 e	30 f	×	×	344 a	32 f
2015	227 f	51 e	391 d	159 bc	273 bc	85 c
2016	211 g	75 d	477 b	183 b	372 a	139 b
2017	293 c	81 d	447 bc	166 b	267 bc	86 c
2018	289 c	118 bc	671 a	405 a	256 cd	150 a
	TPC	ACC_{spec}				
Cultivar	***	***				
Year	***	***				
Interaction	***	***				

Note: ×—no results. TPC—total phenol content; ACC_{spec}—total anthocyanin content determined spectrophotometrically; FSW—fresh skins weight. Different letters in the same columns among years mark significant differences at $p \leq 0.05$. The main effect of the year and cultivar and their interaction, ***—significant at $p \leq 0.001$.

Among nine years, the ACC_{spec} was significantly different—the contents ranged from 30 to 405 mg 100 g^{-1} (Table 4). In 'Hasansky Sladky', the ACC_{spec} ranged from 30 to 138 mg 100 g^{-1} and the highest content was determined in two experimental years out of nine (2010 and 2013). In 'Rondo', ACC_{spec} ranged from 75 to 405 mg 100 g^{-1} and significantly highest content was found in 2018. In 'Zilga', the content ranged from 32 to 150 mg 100 g^{-1} and the highest ACC_{spec} was in one year out of eight (2018). The ACC_{spec} was significantly affected by year, cultivar, and interaction between them ($p \leq 0.001$).

3.3. Antioxidant Activity and Anthocyanins

In all experimental years, TAA in 'Rondo' was significantly higher compared to the other cultivars (ranged between 78 and 88%) (Table 5). TAA in 'Hasansky Sladky' and 'Zilga' did not differ significantly in two out of three years. TAA in 'Hasansky Sladky' ranged from 40 to 53% and in 'Zilga from 53 to 62%. The TAA was significantly affected by cultivar ($p \leq 0.001$) and interaction between cultivar and year ($p \leq 0.01$).

In 2016 and 2018, all individual anthocyanins were significantly higher in 'Rondo' (Table 5). Dp and Cy contents were significantly lower in 'Hasansky Sladky' in all experimental years. Pn did not differ significantly between cultivars 'Hasansky Sladky' and 'Zilga'. Mv and Pt content differed significantly between cultivars and years. Individual anthocyanins were significantly affected by year, cultivar and the interaction between them ($p \leq 0.001$).

Viticultural practice caused significant variability in the content of ACC_{HPLC} and individual anthocyanins (Table 6). TAA was significantly affected by the viticultural practice ($p \leq 0.01$) and interaction of year and viticultural practice ($p \leq 0.05$). ACC_{HPLC} ranged from 447 to 3645 mg 100 g^{-1} in the field and from 1108 to 1618 mg 100 g^{-1} in the tunnel. ACC_{HPLC} and individual anthocyanins were significantly higher in the grapes grown in the field in 2016 and 2018. In 2017, the tunnel grown grapes had higher individual anthocyanin content, Dp by 78%, Cy by 26%, Pt by 81%, Pn by 21%, and Mv by 77%. ACC_{HPLC} and individual anthocyanins were significantly affected by year, cultivar and the interaction between them, except for Dp the viticultural practice did not have a significant effect.

Table 5. The effect of cultivar and year on antioxidant activity and the content of individual anthocyanins' in grapes of 'Hasansky Sladky', 'Rondo', and 'Zilga' (2016–2018) cultivated in field conditions.

Year	Cultivar	TAA %	Dp	Cy	Pt	Pn	Mv
			mg 100 g^{-1} FSW				
2016	'Hasansky Sladky'	40 c	18 c	10 c	20 c	14 b	59 c
	'Rondo'	88 a	311 a	190 a	157 a	302 a	298 a
	'Zilga'	62 b	145 b	90 b	57 b	19 b	75 b
2017	'Hasansky Sladky'	53 b	39 c	23 c	25 c	21 b	41 c
	'Rondo'	78 a	87 b	64 a	33 b	102 a	71 a
	'Zilga'	53 b	111 a	28 b	44 a	21 b	52 b
2018	'Hasansky Sladky'	51 b	111 c	7 c	90 b	21 b	309 b
	'Rondo'	84 a	1189 a	251 a	416 a	333 a	712 a
	'Zilga'	53 b	449 b	107 b	70 b	5 b	72 c
	Year	ns	***	***	***	***	***
	Cultivar	***	***	***	***	***	***
	Interaction	**	***	***	***	***	***

Note: TAA—total antioxidant activity; Dp—delphinidin-3-O-glucoside; Cy—cyanidin-3-O-glucoside; Pt—petunidin-3-O-glucoside; Pn—peonidin-3-O-glucoside; Mv—malvidin-3-O-glucoside: FSW—fresh skins weight. Different letters in columns mark significant differences of means among years according to the cultivars at $p \leq 0.05$. The main effect of the year, cultivar, and the interaction between them, ns—no significance, **—significant at $p \leq 0.01$, ***—significant at $p \leq 0.001$.

Table 6. The effect of viticultural practice on the total antioxidant activity, total anthocyanins, and individual anthocyanins of 'Rondo' grapes (2016–2018).

Year	Viticultural Practice	TAA %	ACC_{HPLC}	Dp	Cy	Pt	Pn	Mv
			mg 100 g^{-1} FSW					
2016	Field	88 a	1581 a	311 a	190 a	157 a	302 a	298 a
	Tunnel	89 a	1108 b	230 b	30 b	113 b	51 b	235 b
2017	Field	78 a	447 b	87 b	64 b	33 b	102 b	71 b
	Tunnel	90 a	1472 a	396 a	86 a	171 a	129 a	306 a
2018	Field	84 a	3645 a	1189 a	251 a	416 a	333 a	712 a
	Tunnel	86 a	1618 b	820 b	12 b	107 b	23 b	177 b
	Year	ns	***	***	**	***	***	***
	Viticultural practice	**	***	ns	***	***	***	***
	Interaction	*	***	***	***	***	***	***

Note: TAA—total antioxidant activity; ACC_{HPLC}—total anthocyanin content determined by chromatographically; FSW—fresh skins weight; Dp—delphinidin-3-O-glucoside; Cy—cyanidin-3-O-glucoside; Pt—petunidin-3-O-glucoside; Pn—peonidin-3-O-glucoside; Mv—malvidin-3-O-glucoside. Different letters in the same columns among years according to the viticultural practice mark significant differences of means at $p \leq 0.05$. The main effects of the year and viticultural practice and their interaction, ns—no significant, *—significant at $p \leq 0.05$, **—significant at $p \leq 0.01$, ***—significant at $p \leq 0.001$.

3.4. Correlations

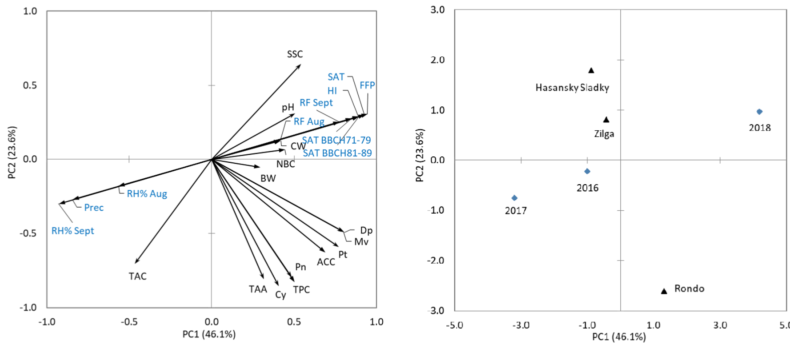
There were significant correlations between climatic data and phenolic compounds (Table 7). In 'Hasansky Sladky', TAA had positive correlation with August RF and negative with August RH. In 'Rondo', TPC had positive correlation with temperature parameters and September RF and negative with precipitation and RH. In 'Hasansky Sladky', TPC correlated only with August RF (positive) and RH (negative). Inversely in 'Zilga', TPC correlated negatively with August RF and positively with precipitation and August RH. In all experimental cultivars, ACC_{HPLC}, Dp, Pt, and Mv had positive correlation with most of the temperature parameters and negative with precipitation and RH. In contrast, Cy in 'Hasansky Sladky' and Cy, Pn in 'Zilga' had negative correlation with temperature parameters. In 'Zilga', Cy and Pt had positive correlation with precipitation and RH.

Table 7. Correlations between phenolic compounds and climatic data for grape cultivars 'Hasansky Sladky', 'Rondo', and 'Zilga' (2016–2018) in field conditions.

	TAA	TPC	ACC_{HPLC}	Dp	Cy	Pt	Pn	Mv
'Hasansky Sladky'								
SAT BBCH 71–79	−0.306 ^{ns}	−0.276 ^{ns}	0.717 *	0.551 ^{ns}	−0.988 **	0.676 *	−0.233 ^{ns}	0.759 *
SAT BBCH 81–89	0.400 ^{ns}	0.505 ^{ns}	0.992 **	0.973 **	−0.586 ^{ns}	0.987 **	0.500 ^{ns}	0.988 **
Year, SAT	−0.087 ^{ns}	−0.029 ^{ns}	0.865 **	0.738 *	−0.925 **	0.834 **	0.001 ^{ns}	0.895 **
HI	−0.211 ^{ns}	−0.167 ^{ns}	0.789 *	0.639 ^{ns}	−0.968 **	0.752 *	−0.130 ^{ns}	0.826 **
August, RF	0.842 **	0.958 **	0.679 *	0.812 **	0.138 ^{ns}	0.712 *	0.910 **	0.633 ^{ns}
September, RF	−0.428 ^{ns}	−0.415 ^{ns}	0.606 ^{ns}	0.423 ^{ns}	−0.996 **	0.560 ^{ns}	−0.366 ^{ns}	0.654 ^{ns}
Frost-free period	0.026 ^{ns}	0.097 ^{ns}	0.920 **	0.815 **	−0.871 **	0.894 **	0.120 ^{ns}	0.943 **
Precipitation	−0.497 ^{ns}	−0.608 ^{ns}	−0.978 **	−0.986 **	0.481 ^{ns}	−0.979 **	−0.595 ^{ns}	−0.966 **
August, RH%	−0.774 *	−0.893 **	−0.800 **	−0.901 **	0.045 ^{ns}	−0.825 **	−0.854 **	−0.763 *
September, RH%	−0.287 ^{ns}	−0.383 ^{ns}	−0.989 **	−0.942 **	0.691 *	−0.977 **	−0.387 ^{ns}	−0.994 **
'Rondo'								
SAT BBCH 71–79	0.583 ^{ns}	0.801 **	0.915 **	0.839 **	0.938 **	0.900 **	0.965 **	0.919 **
SAT BBCH 81–89	0.103 ^{ns}	0.979 **	0.911 **	0.964 **	0.671 *	0.924 **	0.545 ^{ns}	0.916 **
Year, SAT	0.463 ^{ns}	0.923 **	0.983 **	0.945 **	0.918 **	0.977 **	0.894 **	0.988 **
HI	0.534 ^{ns}	0.862 **	0.953 **	0.893 **	0.937 **	0.942 **	0.941 **	0.957 **
August, RF	−0.416 ^{ns}	0.581 ^{ns}	0.379 ^{ns}	0.524 ^{ns}	0.027 ^{ns}	0.411 ^{ns}	−0.171 ^{ns}	0.382 ^{ns}
September, RF	0.638 ^{ns}	0.704 *	0.846 **	0.750 *	0.922 **	0.827 **	0.978 **	0.850 **
Frost-free period	0.390 ^{ns}	0.963 **	0.994 **	0.977 **	0.886 **	0.992 **	0.837 **	0.999 **
Precipitation	−0.014 ^{ns}	−0.949 **	−0.854 **	−0.926 **	−0.584 ^{ns}	−0.871 **	−0.440 ^{ns}	−0.859 **
August, RH%	0.303 ^{ns}	−0.719 *	−0.541 ^{ns}	−0.669 *	−0.198 ^{ns}	−0.569 ^{ns}	−0.007 ^{ns}	−0.545 ^{ns}
September, RH%	−0.198 ^{ns}	−0.994 **	−0.957 **	−0.988 **	−0.755 *	−0.965 **	−0.651 ^{ns}	−0.962 **
'Zilga'								
SAT BBCH 71–79	0.142 ^{ns}	0.149 ^{ns}	0.825 **	0.783 *	−0.838 **	0.954 **	−0.817 **	0.899 **
SAT BBCH 81–89	−0.387 ^{ns}	−0.608 ^{ns}	0.967 **	0.987 **	−0.948 **	0.805 **	−0.965 **	0.340 ^{ns}
Year, SAT	−0.028 ^{ns}	−0.099 ^{ns}	0.936 **	0.912 **	−0.939 **	0.976 **	−0.929 **	0.775 *
HI	0.067 ^{ns}	0.039 ^{ns}	0.881 **	0.847 **	−0.890 **	0.971 **	−0.874 **	0.850 **
August, RF	−0.663 ^{ns}	−0.978 **	0.543 ^{ns}	0.610 ^{ns}	−0.504 ^{ns}	0.196 ^{ns}	−0.547 ^{ns}	−0.390 ^{ns}
September, RF	0.240 ^{ns}	0.294 ^{ns}	0.733 *	0.682 *	−0.752 *	0.913 **	−0.724 *	0.945 **
Frost-free period	−0.114 ^{ns}	−0.223 ^{ns}	0.970 **	0.956 **	−0.968 **	0.963 **	−0.964 **	0.694 *
Precipitation	0.454 ^{ns}	0.700 *	−0.932 **	−0.961 **	0.909 **	−0.730 *	0.931 **	−0.223 ^{ns}
August, RH%	0.632 ^{ns}	0.937 **	−0.685 *	−0.744 *	0.649 ^{ns}	−0.368 ^{ns}	0.688 *	0.220 ^{ns}
September, RH%	0.306 ^{ns}	0.496 ^{ns}	−0.988 **	−0.998 **	0.975 **	−0.873 **	0.985 **	−0.462 ^{ns}

Note: TAA—total antioxidant activity; TPC—total phenol content; ACC_{HPLC}—total anthocyanin content determined by chromatographically; Dp—delphinidin-3-O-glucoside; Cy—cyanidin-3-O-glucoside; Pt—petunidin-3-O-glucoside; Pn—peonidin-3-O-glucoside; Mv—malvidin-3-O-glucoside; ns—non-significant correlation coefficient; significant at $p \leq 0.05$ *, and 0.01 **; SAT—sum of active temperatures (≥10 °C); BBCH—phenological growth stage identification scale; SAT BBCH 71–79—active temperatures of June, July; SAT BBCH 81–89—active temperatures of August, September; HI—Heliothermal Index; RF—radiation flux (W m^2); frost-free period—sum of days with temperature above 0 °C; precipitation—sum of precipitation (mm); RH—relative air humidity (%).

The principal component analysis (PCA) showed that the first principal component (PC1) explained 46% of the total variance in the data, and the second principal component (PC2) explained 24% (Figure 2). The PC1 and PC2 explained 70% of the variance in the data for both Figure 2a,b. The most important determinants of the PC1 were weather-related parameters such as frost free period, HI, SAT, SAT BBCH81–89, SAT BBCH 71–79, RH% Sept and precipitation (Figure 2a). Among grape quality characteristics, Dp, Mv and Pt were the most important determinants of PC1. PC2 was primarily determined by ACC, TPC, TAA, Cy, Pn, and titratable acids content (TAC). It was clearly seen in the PCA map that all polyphenols, especially Dp, Mv, and Pt, were situated in the same area with frost free period, HI, SAT (BBCH 71–79, BBCH 81–89, year) and RF (August, September). Among experimental years, the year 2018 was clearly situated in the same area (Figure 2b). The year 2017 was distinguished in the opposite site, characterized by high precipitation and RH, causing high TAC of fruits. Among cultivars, 'Rondo' was clearly distinguished in the PCA map, situated in the same area with high values of polyphenols and TAA. Other cultivars did not have clear distinction in PCA map.



(**a**) (**b**)

Figure 2. Principal component analysis (PCA) of the structure of biochemical and berry parameters in relation to the cultivar and climatic conditions of experimental years': (**a**) biochemical and berry parameters in relation to the climatic parameters; (**b**) cultivar in relation to the experimental year. BW—berry weight; NBC—berries per cluster; CW—cluster weight; SSC—soluble solids content; TAC—titratable acids content; TAA—antioxidant activity; TPC—total phenol content; ACC—total anthocyanin content determined spectrophotometrically; Dp—delphinidin-3-O-glucoside; Cy— cyanidin-3-O-glucoside; Pt—petunidin-3-O-glucoside; Pn—peonidin-3-O-glucoside; Mv—malvidin-3-O-glucoside; SAT—sum of active temperatures (≥10 °C); BBCH—phenological growth stage identification scale; SAT BBCH 71–79—active temperatures of June, July; SAT BBCH 81–89—active temperatures of August, September; HI—Heliothermal Index; RF—radiation flux (W m^2); FFP—frost-free period, sum of days with temperature above 0 °C; Prec—sum of precipitation (mm); RH—relative air humidity (%).

4. Discussion

4.1. The Effect of Year and Cultivar on Phenolic Compounds

In this 9-year experiment, a high variability in TPC and ACC_{spec} of hybrid grapevine fruits was found as shown in Table 4. Cultivar, year and their interaction significantly affected the contents of these parameters. In addition, correlation and PCA analyses indicated that climatic conditions had an impact on the phenolic compounds in grapes. Warmer and longer vegetation period increased polyphenols content, as seen in years 2018 and more precipitation and higher RH decreased it. The effect was cultivar dependent and 'Rondo' differed significantly from the others. TPC in 'Hasansky Sladky' and 'Zilga' had no correlation with temperature-related parameters, but TPC in 'Rondo' had a correlation with climatic parameters. All the experimental cultivars had a correlation between ACC_{HPLC} and climatic parameters. It may be related to the cultivars' sensitivity to temperature changes. Experimental cultivars had different cluster properties and this could cause variation between cultivars [24]. Berries of 'Rondo' and 'Zilga' are larger and more tightly arranged in the clusters than those of 'Hasansky Sladky'. Additionally, in warmer climates, accumulation of phenolic compounds in *Vitis vinifera* grapes was influenced by environmental factors [5], cultivar [4,6], and *terroir* [7]. The age of the vines also contributed to the years-on-year difference. The vines were three years old at the beginning of the trial, but by the end of the experiment the vines were 11 years old. As the vines grew older, the trunk thickness and the shoots growth intensity changed and that could have affected the results. It is important for vine grower and wine producer to determine the potential of their cultivar of choice for growing.

There was a significant effect of cultivar properties and interaction between year and cultivar on TAA—'Rondo' had the highest TAA as shown in Table 5. It has been found that cultivars with higher TPC had increased TAA as well [8,9]. This was also confirmed in our experiment. In the present study, the content of anthocyanins (delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside, and malvidin-3-O-glucoside) in all tested hybrid grapes depended on the cultivar and growth year as shown in Table 5 and Figure 2. The correlation analyses also indicated that climatic conditions had a correlation with individual anthocyanins and the effect depended on cultivar. Similar results are reported in an experiment made in warmer climate with *Vitis vinifera* cultivars [11], which refers that anthocyanin composition depends on the genetic background of *Vitis* species [10]. Still, the differences in anthocyanin composition are related to the cultivar responses to temperature as well [1]. In all experimental cultivars, Dp, Pt and Mv had a positive relationship with temperature-related parameters and a negative one with precipitation. Relationship of Cy and Pn depended on the cultivar properties. The order of occurrence of individual anthocyanins in the field grown grapes varied among years. For example, in 'Rondo' it was: Dp > Pn > Mv > Cy > Pt in 2016, Pn > Dp > Mv > Cy > Pt in 2017, and Dp > Mv > Pt > Pn > Cy in 2018. Anthocyanins give different colors: Cy—crimson, Pn—magenta, Dp—mauve, Pt and Mv—purple. Dp was the dominant anthocyanin in warmer years and Pn in cooler years. In this experiment, 'Rondo' showed higher potential of phenolic compounds.

4.2. The Effect of Viticultural Practice

The experiment with 'Rondo' showed significant effect of the viticultural practice, year and their interaction on the anthocyanin content as seen in Table 6. The abundance of different individual anthocyanins varied between the field and tunnel grown grapes in two years out of three. In other experiments, different viticultural practices have been shown to affect anthocyanin profile [12,13], as was also confirmed in our experiments. Therefore, it can be concluded that the shade of grape color from the hybrid grapes in a very cool climate conditions vary from year to year and depend on the order of the occurrence of individual anthocyanins. The color of the wines depends in a large extent of the anthocyanins content and distribution. Color is an important factor for evaluating the quality of red wine and is one of the most important factors for consumers when choosing a wine. PCA analysis showed that the technological maturity of grapes also have influenced their phenolic compounds. The technological maturity parameters of experimental cultivars at harvest varied significantly between years and sometimes did not reach the recommended technological maturity for wine making.

Viticultural practice had a significant effect on ACC_{HPLC} in 'Rondo' as shown in Table 6. In 2016 and 2018, ACC_{HPLC} and individual anthocyanins in 'Rondo' were significantly higher in the field cultivated grapes, but in 2017 in the tunnel grown grapes. The contents were significantly influenced by the weather conditions of the experimental years. In 2017, the vegetation period was exceptionally cold and rainy (SAT 1981 °C, HI 888, and precipitation 497 mm). In cooler and rainier years, growing grapes in the tunnel promoted their maturation. In 2016 and 2018, the vegetation period was longer compared to the average, and warmer as well. The year 2018 was exceptionally warm (SAT 2660 °C, HI 1532, and frost-free period 180 days). Elevated temperatures during ripening may reduce the accumulation of anthocyanins and could partly degrade the previously synthesized compounds [14,15]. At *veraison* the temperatures in the tunnel are higher, and day and night temperature fluctuations are greater (min 5 to 6 °C and max 35 to 40 °C) [32]. The differences in growing conditions between the tunnel and the field could have caused variation in compound bud vitality: whether shoots developed from a larger central primary bud, from smaller secondary buds, or from both at the same time. In the field, when the growing season is cooler, the vine primary bud may remain less cold hardy. The second problem is spring frosts. When the primary bud is damaged in cool spring, the smaller secondary or tertiary bud will break, which will greatly affect yield formation.

5. Conclusions

During nine years, significant variability of TPC and ACC in hybrid grapevine fruits was found. 'Rondo' had a higher content of total polyphenols and anthocyanins in most of the experimental years. Variability depended on cultivar and was affected by the climatic parameters of the experimental years. Additionally, the content of individual anthocyanins was affected by the cultivar properties and the climatic parameters. In every year, most abundant individual anthocyanins were malvidin-3-O-glycoside in 'Hasansky Sladky' and delphinidin-3-O-glycoside in 'Zilga'. In 'Rondo' grapes, anthocyanin contents varied from year to year and depended on cultivation site (polytunnel/field). TAA depended on cultivar and it was highest in 'Rondo' every year, therefore it has potential to produce wines rich in antioxidants. Growing 'Rondo' in a high polyethylene tunnel increased the total anthocyanin content during the cooler and rainier year, but decreased it in the warmer year. Individual anthocyanins showed the same tendency. As 'Rondo' is not winter hardy in the Estonian climatic conditions, for that reason it is recommended to be grown in a high polyethylene tunnel.

Author Contributions: Conceptualization, K.K. and M.M.-K.; methodology, K.K. and R.R.; formal analysis, M.M.-K. and P.P.; investigation, K.K., M.M.-K., R.R., L.M. and A.K.; data curation, K.K., M.M.-K., R.R. and A.K.; writing—original draft preparation, M.M.-K.; writing—review and editing, U.M., L.M., P.P. and A.K.; visualization, M.M.-K. and K.K.; supervision, K.K. and U.M.; project administration and funding acquisition, K.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by "Innovation Cluster" of Estonian Rural Development Plan (ERDP Action 16 'Cooperation') 2014–2020, financially supported by European Agricultural Fund for Rural Development (EAFRD).

Acknowledgments: The Estonian Environment Agency and Laboratory of Environmental Physics of Tartu University are gratefully acknowledge for climatic data. Winery Järiste Veinitalu OÜ is gratefully acknowledged for cooperation. European Regional Development Fund is acknowledged for supporting collaboration.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Fernandes De Oliveira, A.; Mercenaro, L.; Nieddu, G. Assessing thermal efficiency for berry anthocyanin accumulation in four different sites and field-growing conditions. *Acta Hortic.* **2017**, *1188*, 181–188. [CrossRef]
2. Zhu, L.; Zhang, Y.; Lu, J. Phenolic contents and compositions in skins of red wine grape cultivars among various genetic backgrounds and originations. *Int. J. Mol. Sci.* **2012**, *13*, 3492–3510. [CrossRef] [PubMed]
3. Wojdyło, A.; Samoticha, J.; Nowicka, P.; Chmielewska, J. Characterisation of (poly)phenolic constituents of two interspecific red hybrids of Rondo and Regent (*Vitis vinifera*) by LC–PDA–ESI-MS QTof. *Food Chem.* **2018**, *239*, 94–101. [CrossRef]
4. Samoticha, J.; Wojdyło, A.; Golis, T. Phenolic composition, physicochemical properties and antioxidant activity of interspecific hybrids of grapes growing in Poland. *Food Chem.* **2017**, *215*, 263–273. [CrossRef] [PubMed]
5. Soubeyrand, E.; Basteau, C.; Hilbert, G.; Van Leeuwen, C.; Delrot, S.; Gomès, E. Nitrogen supply affects anthocyanin biosynthetic and regulatory genes in grapevine cv. Cabernet-Sauvignon berries. *Phytochemistry* **2014**, *103*, 38–49. [CrossRef]
6. Katalinić, V.; Možina, S.S.; Skroza, D.; Generalić, I.; Abramovič, H.; Miloš, M.; Ljubenkov, I.; Piskernik, S.; Pezo, I.; Terpinc, P.; et al. Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 *Vitis vinifera* varieties grown in Dalmatia (Croatia). *Food Chem.* **2010**, *119*, 715–723. [CrossRef]
7. Tarko, T.; Duda-Chodak, A.; Sroka, P.; Satora, P.; Jurasz, E. Polish wines: Characteristics of cool-climate wines. *J. Food Compos. Anal.* **2010**, *23*, 463–468. [CrossRef]
8. Yang, J.; Martinson, T.E.; Liu, R.H. Phytochemical profiles and antioxidant activities of wine grapes. *Food Chem.* **2009**, *116*, 332–339. [CrossRef]
9. Liang, Z.; Cheng, L.; Zhong, G.Y.; Liu, R.H. Antioxidant and antiproliferative activities of twenty-four *Vitis vinifera* grapes. *PLoS ONE* **2014**, *9*, e0105146. [CrossRef]
10. Liang, Z.; Wu, B.; Fan, P.; Yang, C.; Duan, W.; Zheng, X.; Liu, C.; Li, S. Anthocyanin composition and content in grape berry skin in *Vitis* germplasm. *Food Chem.* **2008**, *111*, 837–844. [CrossRef]
11. Ortega-Regules, A.; Romero-Cascales, I.; Lopes-Roca, J.M.; Ros-Garcia, J.M.; Gomez-Plaza, E. Anthocyanin fingerprint of grapes: Environmental and genetic variations. *J. Sci. Food Agric.* **2006**, *86*, 1460–1467. [CrossRef]
12. Downey, M.O.; Harvey, J.S.; Robinson, S.P. The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Aust. J. Grape Wine Res.* **2004**, *10*, 55–73. [CrossRef]

13. Basile, T.; Alba, V.; Gentilesco, G.; Savino, M.; Tarricone, L. Anthocyanins pattern variation in relation to thinning and girdling in commercial Sugrathirteen® table grape. *Sci. Hortic. (Amst.)* **2018**, *227*, 202–206. [CrossRef]
14. Mori, K.; Goto-Yamamoto, N.; Kitayama, M.; Hashizume, K. Loss of anthocyanins in red-wine grape under high temperature. *J. Exp. Bot.* **2007**, *58*, 1935–1945. [CrossRef]
15. Poudel, P.R.; Mochioka, R.; Beppu, K.; Kataoka, I. Influence of Temperature on Berry Composition of Interspecific Hybrid Wine Grape 'Kadainou R-1' (*Vitis ficifolia* var. ganebu × *V. vinifera* 'Muscat of Alexandria'). *J. Jpn. Soc. Hortic. Sci.* **2009**, *78*, 169–174. [CrossRef]
16. Pedastsaar, P.; Vaher, M.; Helmja, K.; Kulp, M.; Kaljurand, M.; Karp, K.; Raal, A.; Karathanos, V.; Püssa, T. Chemical composition of red wines made from hybrid grape and common grape (*Vitis vinifera* L.) cultivars. *Proc. Est. Acad. Sci.* **2014**, *63*, 444–453. [CrossRef]
17. Revilla, E.; Losada, M.; Gutiérrez, E. Phenolic Composition and Color of Single Cultivar Young Red Wines Made with Mencia and Alicante-Bouschet Grapes in AOC Valdeorras (Galicia, NW Spain). *Beverages* **2016**, *2*, 18. [CrossRef]
18. Maante, M. Effect of defoliation on grape maturity parameters. *Sodinink. Darzinink.* **2016**, *35*, 21–35.
19. Rätsep, R.; Karp, K.; Vool, E.; Tõnutare, T. Effect of pruning time and method on hybrid grapevine (*Vitis* sp.) "Hasanski Sladki" berry maturity in a cool climate conditions. *Acta Sci. Pol. Hortorum Cultus* **2014**, *13*, 99–112.
20. Gustafsson, J.G.; Mårtensson, A. Potential for extending Scandinavian wine cultivation. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **2005**, *55*, 82–97. [CrossRef]
21. Karvonen, J. The annual growth cycle of grapevines in southern Finland. *Vitis J. Grapevine Res.* **2014**, *53*, 175–180.
22. Karvonen, J. Phenolic Compounds of Grape Varieties Grown in the Northern Temperate Climate. *Int. J. Agric. Innov. Res.* **2015**, *4*, 311–316.
23. Maante-Kuljus, M.; Rätsep, R.; Mainla, L.; Moor, U.; Starast, M.; Põldma, P.; Karp, K. Technological maturity of hybrid vine (*Vitis*) fruits under Estonian climate conditions. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **2019**, *69*, 706–714. [CrossRef]
24. Vitis International Variety Catalogue VIVC: Passport Data "Hasansky Sladky". Available online: http:/www.vivc.de/index.php?r=passport%2Fview&id=25249 (accessed on 27 February 2020).
25. Vitis International Variety Catalogue VIVC: Passport Data "Zilga". Available online: http://www.vivc.de/index.php?r=passport%2Fview&id=22255 (accessed on 27 February 2020).
26. Vitis International Variety Catalogue VIVC: Passport Data "Rondo". Available online: http://www.vivc.de/index.php?r=passport%2Fview&id=14308 (accessed on 27 February 2020).
27. Lorenz, D.H.; Eichhorn, K.W.; Bleiholder, H.; Klose, R.; Meier, U.; Weber, E. Growth stages of the grapevine: Phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. vinifera)—Codes and descriptions according to the extended BBCH scale. *Die Weinwiss. Vitic. Enol. Sci.* **1994**, *49*, 66–70. [CrossRef]
28. Huglin, P. A new method of evaluating the heliothermal possibilities in the environment of grape culture. *C. R. Acad. Agric. Fr.* **1978**, *64*, 1117–1126.
29. Wrolstad, E.; Ronald Acree, E.T.; Decker, A.E.; Penner, H.M.; Reid, S.D.; Smith, J.; Steven Schwartz, F.; Charles Shoemaker, D.; Sporns, P. *Handbook of Food Analytical Chemistry. Pigments, Colorants, Flavors, Texture, and Bioactive Food Components*; John Wiley & Sons: Hoboken, NJ, USA, 2005. [CrossRef]
30. Huang, D.; Boxin, O.U.; Prior, R.L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* **2005**, *53*, 1841–1856. [CrossRef]
31. Lambert, M.; Meudec, E.; Verbaere, A.; Mazerolles, G.; Wirth, J.; Masson, G.; Cheynier, V.; Sommerer, N. A high-throughput UHPLC-QqQ-MS method for polyphenol profiling in rosé wines. *Molecules* **2015**, *20*, 7890–7914. [CrossRef]
32. Maante-Kuljus, M.; Vool, E.; Mainla, L.; Starast, M.; Karp, K. Berry quality of hybrid grapevine (*Vitis*) cultivars grown in the field and in a polytunnel. *Agric. Food Sci.* **2019**, *28*, 137–144. [CrossRef]

CURRICULUM VITAE

First name:	Mariana
Last name:	Maante-Kuljus (Maante)
Date of birth:	March 15th 1989
E-mail:	mariana.maante-kuljus@emu.ee

Education

2014 – 2021	Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, PhD studies in Agricultural Sciences
2012 – 2014	Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, MSc studies in Horticulture (*cum laude*)
2009 – 2012	Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, BSc studies in Horticulture

Employment

Since 09.2019	Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Chair of Horticulture, assistant
09.2018 – 2019	Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Chair of Horticulture, junior researcher

Professional training

International Summer Course in Innovative Education towards Sustainable Food Systems. E-learning 15.05.-15.07.2017; summerschool 23.07.-06.08.2017 Warsaw University of Life Sciences, Poland.

Research interests

Biosciences and environment, agricultural sciences, phytotechny, horticulture, crop protection, phytopathology

Dissertations supervised

Reemet Tanberg, Master's Degree, 2018,(sup) Mariana Maante-Kuljus, Reelika Rätsep; Phenolic compounds in grapevine (*Vitis*) yield depending on growing environment, Estonian University of Life Sciences.

Funding and projects

T190071PKAN (616118790022) "ORGANIC BLUEBERRY. Mitigating the risks of climate change in the production of organic blueberries (1.01.2019−31.12.2022)", Leila Mainla, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Chair of Horticulture.

ETF9363 "Effect of the organic cultivation technologies on content of bioactive compounds in blueberry and grapevine fruits (1.01.2012−30.06.2016)", Ele Vool, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences. (Completed)

ELULOOKIRJELDUS

Eesnimi:	Mariana
Perekonnanimi:	Maante-Kuljus (Maante)
Sünniaeg:	15. märts 1989
E-mail:	mariana.maante-kuljus@emu.ee

Haridus:

2014 – 2021	Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, aianduse õppetool, doktoriõpe põllumajanduse erialal
2012 – 2014	Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, aianduse osakond, magistriõpe aianduse erialal
2009 – 2012	Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, aianduse osakond, bakalaureuseõpe põllu- ja aiasaaduste tootmise erialal

Teenistuskäik:

09.2019 –…	Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Aianduse õppetool, assistent
09.2018-2019	Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Aianduse õppetool, nooremteadur

Enesetäiendus ja koolitused

Rahvusvaheline suvekool "Innovaatiline haridus jätkusuutlikuks toidutootmiseks" E-õpe 15.05.-15.07.2017; suvekool 23.07.-06.08.2017 Varssavi Põllumajandusülikool, Poola.

Teadustöö põhisuunad

Bio- ja keskkonnateadused, põllumajandus-teadus, taimekasvatus, aiandus, taimekaitsevahendid, taimehaigused

Juhendatud väitekirjad

Reemet Tannberg, magistrikraad, 2018, (juh) Reelika Rätsep; Mariana Maante, Fenoolsete ühendite sisaldus viinapuu (Vitis) saagis sõltuvalt kasvukeskkonnast, Eesti Maaülikool.

Uurimisprojektides osalemine

T190071PKAN "MAHEMUSTIKAS. Kliimamuutustest tulenevate riskide maandamine mahemustika tootmisel (1.01.2019−31.12.2022)", Leila Mainla, Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Aianduse õppetool.

ETF9363 (ETF9363) "Mahekasvatustehnoloogiate mõju mustika ja viinamarja viljade bioaktiivsete ühendite sisaldusele (1.01.2012−30.06.2016)", Ele Vool, Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut.

LIST OF PUBLICATIONS

1.1. Articles indexed by Thomson Reuters Web of Science or Scopus

Maante-Kuljus, M., Rätsep, R., Moor, U., Mainla, L., Põldma, P., Koort, A., Karp, K. 2020. Effect of Vintage and Viticultural Practices on the Phenolic Content of Hybrid Winegrapes in Very Cool Climate. *Agriculture*, 10 (5), doi:169.10.3390/agriculture10050169.

Rätsep, R., Karp, K, **Maante-Kuljus, M.**, Aluvee, A., Bhat, R. 2020. Polyphenols and Resveratrol from Discarded Leaf Biomass of Grapevine (*Vitis* sp.): Effect of Cultivar and Viticultural Practices in Estonia. *Agriculture*, 10 (9), doi:10.3390/agriculture10090393.

Koort, A., Starast, M., Põldma, P., Moor, U., Mainla, L., **Maante-Kuljus, M.**, Karp, K. 2020. Sustainable Fertilizer Strategies for Vaccinium corymbosum x V. angustifolium under Abandoned Peatland Conditions. *Agriculture*, 10 (4), doi:121.10.3390/agriculture10040121.

Maante-Kuljus, M., Rätsep, R., Mainla, L., Moor, U., Starast, M., Põldma, P., Karp, K. 2019. Technological maturity of hybrid vine (*Vitis*) fruits under Estonian climate conditions. Acta *Agriculturæ Scandinavica, Section B - Soil & Plant Science*, 69 (8), 706−714. doi:10.1080/09064710.2019.1641547.

Maante-Kuljus, M., Vool, E., Mainla, L., Starast, M., Karp, K. 2019. Berry quality of hybrid grapevine (*Vitis*) cultivars grown in the field and in a polytunnel. *Agricultural and Food Science*, 28 (3), 137−144. doi:10.23986/afsci.76822.

Maante, M., Vool, E., Rätsep, R., Karp, K. 2015. The effect of genotype on table grapes soluble solids content. *Agronomy Research* 13(1): 141-147.

1.2. Articles published in International peer-reviewed journals not indexed by Thomson Reuters or Scopus

Maante, M., Vool, E., Karp, K. 2016. Effect of Defoliation on Grape Maturity Parameters. *Sodininkyste ir Darzininkyste* (Horticulture and Vegetable Growing), 35 (1-2), 21−35.

3.5. Articles/ presentations published in local conference proceedings

Maante, M., Vool, E., Rätsep, R., Karp, K. 2015. Viinamarjade kvaliteedi mõjutamise võimalused. *Agronoomia 2015*: 185-190. Tartu: Ecoprint, 2015.

5.2. Conference abstracts

Maante, M., Vool, E., Karp, K. 2016. The effect of protected cultivation and open field conditions on grape quality in cooler climate. *The Food Factory I Barcelona Conference*: 88. Spain.

6.3. Popular science articles

Vool, E., **Maante, M.** 2016. Viinamarjakasvatuses edendamine jaheda kliimaga piirkondades. *Aiandusfoorum* 2016 (6−8). Eesti Põllumajandus-Kaubanduskoda.

VIIS VIIMAST KAITSMIST

HELIS LUIK-LINDSAAR
EESTI PÕLLUMAJANDUSETTEVÕTETE JA MAAPIIRKONNA KOHALIKE OMAVALITSUSTE TEHNILISE EFEKTIIVSUSE HINDAMINE ANDMERAJA ANALÜÜSI MEETODIGA
EVALUATION OF TECHNICAL EFFICIENCY IN FARMS AND RURAL MUNICIPALITIES IN ESTONIA USING DATA ENVELOPMENT ANALYSIS
Professor **Rando Värnik** ja vanemteadur **Ants-Hannes Viira**
21. detsember 2020

KAIRI MAILEHT
FÜTOPLANKTON JÄRVEDE SEISUNDI INDIKAATORINA
PHYTOPLANKTON AS ECOLOGICAL QUALITY INDICATOR OF LAKES
Professor Emeritus **Ingmar Ott**, juhtivteadur **Peeter Nõges**
19. veebruar 2021

PAUL FRIDTJOF MÕTSKÜLA
ELEKTRO- JA PILTDIAGNOSTIKA TÄIENDAVAD RAKENDUSED KOERTE SÜDAMEHAIGUSTE DIAGNOOSIMISEL NING PROGNOOSIMISEL
CONTRIBUTION TO THE DIAGNOSIS AND PROGNOSIS OF CANINE CARDIAC DISEASE THROUGH ELECTRODIAGNOSTICS AND DIAGNOSTIC IMAGING
Professor **Toomas Orro**, Professor **Virginia Luis Fuentes** (The Royal Veterinary College, UK), Professor **David Connolly** (The Royal Veterinary College, UK) ja doktor **Ranno Viitmaa**
8. märts 2021

JONATHAN MARTIN WILLOW
TIAKLOPRIIDI, TAIMSETE EETERLIKE ÕLIDE JA KAHE-AHELALISE RNA RAKENDAMISE VÕIMALUSED HIILAMARDIKATE KESKKONNASÄÄSTLIKUS TÕRJES
EXAMINING THIACLOPRID, ESSENTIAL OILS AND DOUBLE-STRANDED RNA FOR POTENTIAL USE IN BIOSAFE MANAGEMENT OF POLLEN BEETLE
Professor **Eve Veromann**, professor **Guy Smagghe**
12. aprill 2021

MARIA SOONBERG
GRUPEERIMISE MÕJU PIIMALEHMADE KÄITUMISELE JA HEAOLULE
REGROUPING EFFECTS ON BEHAVIOUR AND WELFARE OF DAIRY COWS
Professor **David Arney**, doktor **Marko Kass** ja professor **Tanel Kaart**
26. aprill 2021

ISSN 2382-7076
ISBN 978-9949-698-74-5 (trükis)
ISBN 978-9949-698-75-2 (pdf)